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(57) Abstract

The S-locus of *Brassica* contains the genetic information that encodes for self-incompatibility. In its first aspect, it is directed to an isolated gene, the SRK-910 gene, that segregates with the self-incompatibility phenotype. In its second aspect, the present invention is directed to an isolated cDNA that corresponds to the isolated gene and that has 2749 nucleotides. The isolated cDNA of the present invention encodes for a protein, i.e., the S-locus receptor kinase-910 protein ("the SRK-910 protein") which is also a part of the present invention. The SRK-910 protein has 858 amino acids and is encoded for by the first 2755 nucleotides of the isolated cDNA of the present invention. The present invention is also directed to an oligonucleotide probe that is capable of distinguishing the SRK-910 gene from partially homologous genes at the S-locus that encode for the S-locus glycoproteins. The present invention is also directed to a transfer vector comprising the cDNA for the SLG-910 allele in combination with the cDNA of Claim 1. Finally, the present invention is also directed to a method for conferring the self-incompatible phenotype on a self-compatible plant comprising transferring the disclosed transfer vector into a plant that is capable of assimilating the transfer vector and expressing self-incompatibility.

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S-LOCUS RECEPTOR KINASE GENE IN A SELF-INCOMPATIBLE *Brassica* NAPUS LINE

Background of the Invention

a. Field of the Invention

The present invention is directed to an isolated gene from the S-locus of *Brassica*, i.e., the S-locus receptor kinase 910 (SRK-910) gene. More particularly, the present invention is directed to an isolated cDNA sequence which encodes for the SRK-910 protein. Th presence of the SRK-910 gene at the S-locus of *Brassica* is associated with expression of the self-incompatibility (SI) phenotype. The present invention is also directed to the recombinant SRK-910 protein. The present invention is further directed to specific cDNA probes that are capable of hybridizing with the SRK-910 gene and the isolated cDNA sequence. The present invention is useful because it permits the rapid identification of *Brassica* progeny that manifest the self-incompatibility (SI) phenotype.

b. Background of the Invention

Self-incompatibility is an interesting example of cell-cell recognition in plants. There are at least fifty different alleles in *Brassica* and in each case the stigma papillae cells must be able to differentiate between self-pollen and pollen derived from parents carrying different S-alleles. Once this recognition event occurs, it sets in motion a train of physiological events that prevents the germination of self-incompatible pollen, while allowing the germination and subsequent fertilization by self-compatible pollen even when both types are present on the stigma surface (Gaude and Dumas, 1987).

In animal cells, this type of recognition event is often mediated by plasma membrane-associated receptor kinases (Cadena & Gill, 1992). In these cases, the receptor binds to the extracellular ligand molecule and the binding stimulates a change in conformation of the kinase domain thereby stimulating kinase activity which

regulates the subsequent changes in gene expression (Cantley et. al., 1991; Karen, 1992). In plants, much less is known about this type of signal recognition process in general, and in the self-incompatibility response in particular.

Signal transduction by receptor kinases occurs in many aspects of cell growth, development and differentiation (Karin, 1992; Cadena & Gill, 1992). The majority of receptor kinases characterized to date have been found to specifically phosphorylate tyrosine residues (Ullrich & Schlessinger, 1990). Mutations in these types of receptors have also been implicated in oncogenesis (Aaronson, 1991; Cantley et al. 1991). Recently, there have been a few reports of other receptor kinases with homologies to serine/threonine cytoplasmic kinases. One of these receptor kinases has been shown to possess serine/threonine phosphorylation activity (Lin et al., 1992), while another displays serine, threonine and tyrosine kinase activity (Douville et al., 1992). In plants, there is very little known about the role of receptor kinases in signal transduction. There have been three reports on the isolation of plant receptor kinases (Walker & Zhang, 1990; Stein et al., 1991; Tobias et al., 1992). Based on sequence homology only, these genes appear to encode serine/threonine kinases. One of these receptor kinases, SRK-6, has been implicated in the self-incompatibility system of *Brassica oleracea* (Stein et al., 1991).

Self-incompatibility in *Brassica* is controlled by a single dominant genetic locus called the S-locus (Bateman, 1955). The sporophytic nature of this incompatibility system results in the pollen phenotype being derived from the genotype of the diploid pollen parent and not from the haploid pollen genotype. This is hypothesized to occur by the deposition of an S-factor in the exine (outer coat) of the pollen grain by the anther tapetum (parental tissue) during pollen development (de Nettancourt, 1977). When a pollen grain lands on the

stigma surface, the action of the S-locus results in a block in fertilization if the same S-allele is present in the pollen parent and the pistil. The response is very rapid, and for the stronger alleles, leads to a block in pollen hydration or some hydration and germination, and an inability to penetrate the stigma barrier (Zuberi & Dickinson, 1985; Gaude & Dumas, 1987). There are multiple alleles at the S-locus and it has ben estimated that in *B. oleracea* there are nearly 50 different all les (Ockendon 1974, 1982). In heterozygous plants, the majority of *B. oleracea* S-alleles have been found to b dominant, codominant, or recessive to the second allele in a non-linear arrangement dependent on the allele combinations. A few alleles, called pollen recessive alleles, have been shown to be always recessive to other S-alleles in the pollen (Thompson & Taylor, 1966). Both of the diploid *Brassica* species, *B. campestris* and *B. oleracea*, possess this self-incompatibility system, while *B. napus*, an allotetraploid composed of the *B. campestris* and *B. oleracea* genomes, generally occurs as a self-compatible plant (Downey & Rakow, 1987). There are a few naturally occurring self-incompatible *B. napus* lines (Olsson, 1960, Gowers, 1981), and self-incompatible lines have also been generated by introgressing an S-locus from *B. campestris* (Mackay, 1977).

Initial studies on the *Brassica* self-incompatibility system have shown that there is an abundant soluble glycoprotein present in the cell wall of the stigma papillae cells associated with this response (Nasrallah et al., 1970; Hinata & Nishio, 1978; Kandasamy et al., 1989). Several genes for these S-locus glycoproteins ("SLG") have been cloned and characterized (Nasrallah et al., 1987; Trick & Flavell, 1989; Dwyer et al., 1991). Among the alleles associated with a strong incompatibility phenotype, there is greater than 80% homology at the DNA level (Dwyer et al., 1991). The weak pollen recessive alleles are also highly homologous to each other, but only about 70% homologous to the first

group of phenotypically strong alleles (Scutt & Croy, 1992). Transformation of a self-compatible *B. napus* line with these SLG alleles does not produce a self-incompatibility phenotype (Nishio et al., 1992). Recently, a second gene at the S-locus has been cloned from *B. oleracea*. This second gene, a S-locus receptor kinase gene (SRK-6), shows sequence homologies at its N-terminal end to SLG genes and at its C-terminal end to serine/threonine kinases (Stein et al., 1991).

It is an object of the present invention to find and isolate one or more genes that are associated with the self-incompatibility phenotype of *Brassica*. It is a further object of the present invention to characterize the isolated gene and to develop probes that would enable one to rapidly screen the progeny of cross fertilizations between *Brassica* species for the self-incompatibility (SI) phenotype.

SUMMARY OF THE INVENTION

The present invention has multiple aspects. In its first aspect, it is directed to an isolated gene, the SRK-910 gene, from the S-locus of *Brassica*. The presence of the SRK-910 gene at the S-locus of *Brassica* is associated with the presence of the self-incompatibility (SI) phenotype in that species. In its second aspect, the present invention is directed to an isolated cDNA (SEQ ID No. 1) that corresponds to an allele of the self-incompatibility locus (SI-locus) of *Brassica*. The isolated cDNA (SEQ ID No. 1) has 2749 nucleotides and the sequence in Figure 4. The isolated cDNA encodes for the S-locus receptor kinase-910 protein ("the SRK-910 protein"), which plays a role in the self-incompatibility of *Brassica*. The number "910" refers to the 910 gene, which was established to segregate with the W1 SI phenotype (see Goring et al. (1992a)). The SRK-910 protein (SEQ ID No. 2) has 858 amino acids (Figure 9) and is encoded for by the first 2574 nucleotides of the isolated cDNA (SEQ ID No. 1) of the present invention.

The present invention is also directed to a DNA probe that is capable of hybridizing within the nucleotide sequence of Figure 4 but not with the nucleotide sequences of partially homologous genes at the S-locus that encoding for the SLG glycoproteins. The DNA probe of the present invention is a member of a group of four oligonucleotide probes, as shown in Figure 12 herein and having SEQ ID Nos. 5-8.

In another aspect, the present invention is directed to a vector comprising the isolated cDNA (SEQ ID No. 1) of the present invention. Preferably, the vector further comprises the isolated cDNA corresponding to the SLG-910 allele which is described in Goring et al. (1992a). Most preferably, the vector is a transfer vector.

In yet another aspect, the present invention is directed to a method for conferring the self-incompatible (SI) phenotype on a self-compatible (SC) plant. The method comprises transferring the transfer vector of the present invention into a self-compatible plant, plant tissue or plant protoplast that is capable of assimilating the transfer vector and expressing self-incompatibility.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A is a composite of the regions cloned from the SRK-910 gene. The dotted portion of the Coding Region represents the receptor domain. The cross hatched portion of the Coding Region represents the kinase domain.

Figure 1B is a 800 bp genomic fragment of the SRK-910 gene isolated from the SLG-homology domain using the general self-incompatibility primers SI-1 (SEQ ID No. 4) and SI-2 (SEQ ID No. 5), both of FIGURE 10.

Figure 1C is a genomic fragment encompassing the 5' end isolated by inverse PCR using the SRK-910 specific primers: primer 2 (SEQ ID No. 6) and primer 3 (SEQ ID No. 7), both of Figure 12.

Figure 1D is a cDNA clone composed of th 3' nd isolated by 3' RACE using the SRK-910 specific primers:

primer 1 (SEQ ID No. 5) and primer 3 (SEQ ID No. 7), both of Figure 12.

Figure 2 is an analysis of the SRK-910 message for intron splicing. The kinase domain was amplified from various samples and digested with *Alu* I to look for th presence of introns. Sources of DNA for PCR amplification are as follows: Lane 1: Wl genomic DNA; Lane 2: a reconstructed SRK-910 clone carrying the correct coding region; Lanes 3 and 4: amplified cDNA directly from stigma cDNA; Lanes 5-7: altered SRK-910 cDNA clones 10, 24, and 26; Lane 8: 1 kb ladder (BRL). The altered *Alu* 1 fragments in the cDNA clones are marked by dots (Lanes 5-7).

Figure 3 is a blot of genomic DNA taken from F2 plants derived from a cross between an SI plant homologous for Wl (Lane 1) and an SC plant homologous for Westar (Lane 3) to produce a heterologous Wl X Wester Fl (Lane 2) which was then self pollinated to produce an F2 population (Lanes 4-19). The genomic DNA was digested with Hind III and hybridized to the entire SRK-910 coding region. The plants in Lanes 1, 2 and 4-11 are self-incompatible (SI) while the plants in Lanes 3 and 12-19 are self-compatible (SC).

Figure 4 is the nucleotide sequence and predicted amino acid sequence of the SRK-910 gene (SEQ ID No. 1). The underlined sections represent the signal peptide and transmembrane domain, respectively. Conserved cysteine residues are marked by a dash above the amino acid residue. Potential N-glycosylation sites are represented by bold-italic type. The nucleotide sequence has been submitted to GenBank, IntelliGenetics, Inc., Mountain View, CA., Accession No. M97667.

Figure 5 is an analysis of the SRK-910 sequence. At the top of the figure, there is a Kyte hydropathy plot of the predicted amino acid sequence generated by PROSIS software (using a window value of 10). Increased hydrophobicity is indicated by positive values. Below the plot, the domains of the SRK-910 protein are

illustrated and compared. A comparison of amino acid homology is shown between the SRK-910 receptor and its SLG-910 counterpart. The SRK-910 receptor and kinase domains are also compared to SRK-6 and SRK-2 from *B. oleracea* (Stein et al., 1991), to ARK-1 from *Arabidopsis* (Tobias et al., 1992); and to ZMPK-1 from corn (Walker & Zhang, 1990). DNA homologies for the SLG-910 and SRK-6 genes (alleles) are shown in brackets.

Figure 6 represents an alignment of kinase domains from plant receptor kinases. Using conventional single letter designations (Table 1) for amino acids, the amino acid sequence of the SRK-910 kinase domain is compared to that of SRK-6 and SRK-2 from *B. oleracea* (Stein et al., 1991), ARK-1 from *Arabidodpsis* (Tobias et al., 1992); and ZMPK-1 from corn (Walker & Zhang, 1990). Capital letters indicate amino acids that are the same as the SRK-910 protein while differences are denoted by small letters. As defined by Hanks et al. (1988), the kinase sequences have been divided into 11 domains. The amino acids that are conserved in protein kinases are shown in the top line. The bold type represents amino acid that ar absolutely conserved and the regular type represents conserved amino acid groups as defined by Hanks et al. (1988). The two underlined regions represent consensus sequences found in serine/threonine kinases.

Figure 7 is an analysis of SRK-910 kinase activity in E. coli. Figures 7A and 7B represent SDS-PAGE gel containing glutathione S-transferase ("GST") fusion proteins extracted with glutathione agarose beads and tested for kinase activity (autophosphorylation) by the addition of γ^{32}P-ATP. A coomassie blue stain of the gel is shown in A and an autoradiogram to detect phosphorylated proteins is shown in B. Sigma brand SDS molecular weight markers (M) are shown on the left. In both Figures 7A and 7B, the lanes are as follows: Lane 1: HB101 extract with no plasmids; Lanes 2 and 3: control plasmids without an SRK-910 insert; Lanes 4 and 6: wt SRK-910 kinase domain fused to the two different vectors;

Lanes 5 and 7: SRK-910 kinase domain carrying a mutated lysine fused to the two different vectors. The full length fusion proteins are marked by dots.

Figure 7C is a phosphoamino acid analysis of th "protein A-GAST-(SRK-910) receptor kinase" ("AGST-kinase") fusion protein. Hydrolysed amino acids were separated by two-dimensional thin-layer electrophoresis. The positions of the control phosphoamino acids visualized by ninhydrin are marked by the dotted circles. pY=phosphotyrosine, pT=phosphothreonine, pS=phosphoserine.

Figure 8A is an RNA blot analysis of the SRK-910 transcripts in poly A+ RNA extracted from different tissues. The anther and pistil samples were extracted from different bud sizes with Lane 1 = 2 to 3 mm; Lane 2 = 4 to 5 mm; and Lane 3 = 6 to 7 mm in length. After hybridization with the SRK-910 probe, the RNA blot was reprobed with an *Arabidopsis* actin clone to show that RNA was present in all lanes. The presence of some 18S (1.8 kb) and 25S (3.4 kb) ribosomal RNA in the poly A+ RNA preps allowed for their positions to be marked (on the right).

Figures 8B and 8C represent a PCR analysis of SRK-910 transcripts. First strand cDNA synthesized from total RNA samples were amplified for 25 cycles with the SRK-910 specific primers, primer 3 (SEQ ID No. 3) and primer 4 (SEQ ID No. 4) each having 20 bases. Ethidium bromide stain of the gel is shown in Figure 8B. A DNA blot of the gel hybridized to the SRK-910 probe is shown in Figure 8C. The anther and stigma (plus style) samples (Lanes 1 to 4) were extracted from different bud sizes ranging from approximately 4 to 7 mm in length. A 1 kb ladder (BRL) was used as the molecular weight markers.

Figure 9 is the amino acid sequence of the SRK-910 protein using one letter symbols (Table 1).

Figure 10 illustrates the general self-incompatibility primers used in the isolation of the SRK-910 cDNA. SI-1 (SEQ ID No. 4) and SI-2 (SEQ ID No. 3)

represent conserved regions shown in published SLG sequences. Primers were made from these sequences and used in the PCR reaction to amplify the W1 associated bands from genomic DNA. The adaptor (SEQ ID No. 10) and dT_n-adaptor (SEQ ID No. 9) primers were designed according to Frohman et al. (Proc. Natl. Acad. Sci. 85:8998-9002, 1988), with different restriction enzyme sites incorporated into the adaptor primer.

Figure 11 illustrates a genomic DNA blot analysis of related SLG sequences. Genomic DNA samples were digested with Hind III, hybridized with the A14 cDNA and washed at reduced stringency to detect cross hybridizing genes. The genomic DNA samples are the SC Westar (Lane 1) , SI W1 (Lanes 2 and 3), and progeny from two different 3-way crosses involving W1 and various SC canula lines. Lanes 3-6 represent one cross, and Lanes 7-19 represent the second cross. The plants were tested for self-incompatibility by seed set. Lanes 4, 6 and 8-14 are SI, and Lanes 5, 7 and 15-19 are SC. The arrows mark two cross-hybridizing bands which are only present in the genomic DNA samples from SI plants.

Figure 12 provides the nucleotide sequence and location for the SRK-910 specific primers, namely "primer 1" (SEQ ID No. 5), "primer 2" (SEQ ID No. 6), "primer 3" (SEQ ID No. 7), and "primer 4" (SEQ ID No. 8). The primers were chosen by comparing the partial SRK-910 genomic sequence to published SLG and SRK sequences and selecting the variable regions. Compare for example Figure 6.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is directed to an isolated gene, the SRK-910 gene, which was isolated from the SI locus of the self-incompatible canola line, *Brassica napus ssp. oleifera* W1. The S-locus in the W1 line gives a very strong self-incompatibility response and provides a useful line and S-allele for producing hybrid canola lines that exhibit self-incompatibility.

In its second aspect, the present invention is directed to an isolated cDNA i.e., the cDNA (SEQ ID No. 1) of Figure 4 having 2749 nucleotides. The cDNA of the present invention (SEQ ID No. 1) was isolated from the self-incompatible canola line (*Brassica napus ssp. oleifera*) W1 which was produced by introgressing a *B. campestris* S-locus into the self-compatible Westar canola cultivar.

The present invention is further directed to nucleotides 1-2574 of the isolated cDNA which encode for the S-locus receptor kinase 910 protein ("the SRK-910 protein"). The SRK-910 protein (SEQ ID No. 2) comprises the sequence of 858 amino acids of Figure 9.

The preparation of the W1 line is fully described in Goring et al. (1992a). By way of summary, WI is a self-incompatible *B. napus ssp. oleifera* (canola) cultivar derived from the introgression of a *B. campestris* S-locus into the self-compatible (SC) canola Westar line. DNA blot analysis of W1 genomic DNA with the SLG-A14 allele isolated from another canola line revealed two cross--hybridizing *Hind* III bands of 3.6 kb and 6.5 kb, respectively (see Goring et al. (1992a)). A gene corresponding to the 6.5 kb band was isolated and characterized as described in Goring et al. (1992a). The gene that was isolated from the 6.5 kb band was found to encode for a highly expressed SLG-910 allele which segregates with W1 self-incompatibility.

The present invention is directed to our isolation and cloning of the S-locus receptor kinase (SRK) 910 gene from the above mentioned 3.6 kb band. In the present invention, we have determined that the SRK-910 gene also segregates with W1 self-incompatibility.

MATERIALS AND METHODS

Standard chemical materials and standard molecular biological methods were used in this invention. Modifications to the protocols were made as described herein.

Genomic DNA Extraction

Genomic DNA was extracted from leaves using a modified version of Fedoroff et al. (Genet. 2:11-29, 1983.) Approximately 1g of tissue was homogenized in a mortar and pestle in the presence of liquid nitrogen. Six milliliters (mls) of extraction buffer (8M urea, 350mm NaCl, 50mm Tris-Cl, pH 7.5, 20mM EDTA, 2% Sarcosine) were added to the tissue and grinding was continued until the materials were thawed. The mixture was then transferred to an 15ml polypropylene tube, and 0.6ml 10% SDS and 6ml phenol/chloroform/isoamyl-alcohol (75:24:1) were added. The mixture was gently shaken for 10 min and separated by centrifugation. The supernatant was then extracted with 1 volume of phenol/chloroform/isoamyl-alcohol (25:24:1) followed by an extraction with chloroform/isoamyl-alcohol (24:1). The nucleic acids were precipitated with a 1/10th volume of 3M sodium acetate and 2 volumes ice-cold ethanol. Nucleic acid was then resuspended in 2ml 10mM Tris-Cl, pH 8.0, 45mM EDTA and treated with 60μg RNAse A at 37°C for 30 min. The DNA was ethanol-precipitated and resuspended in 100-200μl TE (10mM Tris, 1mM EDTA, pH 7.5). A scaled down version which involved grinding one leaf in an eppendorf tube was utilized for the F2 plants. For the purpose of rapidly screening seedlings for the presence of S-alleles, DNA was prepared by the method of Edwards and Thompson, (Nucl. Acids. Res. 19:1349, 1991).

Genomic DNA Blots

Approximately 5 to 10μg of genomic DNA was digest d to completion with the restriction endonuclease Hind III (Bethesda Research Laboratories, Bethesda, MD). Digest d DNA was then fractionated through a 0.7% agarose gel, and transferred to a Zetabind™ membrane (Cuno Labs Inc., Meridien, CT) by blotting in 20X SSC (20X SSC = 3M sodium acetate, 0.3M Na_3 citrate $\cdot 2H_2O$. After drying, the membrane was prewashed in 0.1X SSC, 0.5% sodium dodecylsulfate solution (SDS) for 30 min. at 60°C. The membranes were prehybridized at 42°C in 5X SSPE, 10X

Denhardt's (10X Denhardt's=1g Ficoll 400, 1g polyvinylpyrrolidone, 1g bovine serum albumin [Pentax fraction V], in 500ml of distilled water), 0.5% SDS for approximately one hour, hybridized overnight at 42°C in 50% formamide, 10% dextran sulfate, 5X SSPE, 0.5% SDS, and 50μg/μl sheared salmon sperm DNA. Filters were then washed at 68°C to 70°C in 0.1X SSC, 0.1% SDS. Hybridization probes consisting of full length cDNAs were digested with the appropriate restriction endonucleases to excise the cDNA from the vector. The excised cDNA was separated from the vector by electrophoresis on an agarose gel. Probes were labelled by random-priming using the method of Feinberg & Vogelstein, (Anal. Biochem. 132:6-13, 1983.)

DNA Sequencing

The 5' and 3' cDNA end clones were partially sequenced using dideoxy sequencing method of Sanger and the Sequenase enzyme (United States Biochemicals, Cleveland Ohio) (Sanger, F., et al., Proc. Natl. Acad. Sci. U.S.A. 74:5463-5467). To sequence the full length cDNA clones, deletions were made using exonuclease III and Mung Bean nuclease according to the procedure in the Stratagene kit (Stratagene, LaJolla CA). Overlapping deletions were sequenced for both strands. All DNA and protein sequence analysis was performed on the DNASIS and PROSIS software. (Pharmacia, Piscataway, NJ).

RNA extraction

Total RNA was extracted from about 100-200mg of tissue using the method of Jones et al. (EMBO J. 4:2411-2418, 1985.) 10 to 30μg of RNA was fractionated through a 1.2% formaldehyde-agarose gel (Sambrook et al., A Laboratory Manual. 2nd ed. Cold spring Harbor Laboratory Press, 1989) and transferred to Zetabind™ membrane (Cuno Labs. Inc.) in 20X SSC. Hybridization and washing conditions were the same as used for the genomic blots.

DESCRIPTION

Isolation Of The SRK-910 Gene In The W1 Line

The initial characterization of the W1 line involved hybridization of the A14 cDNA to a genomic DNA blot washed with reduced stringency at 50°C in 1XSSC 0.1% sodium dodecyl sulfate (SDS), which allows hybridization to sequences having about 65% homology and greater. Under these conditions, multiple bands could be detected in both SI and SC plants as illustrated (Figure 11). However, two hybridizing bands were found to be present in W1 genomic DNA (Figure 11, Lanes 2 and 3) and in SI plants (Figure 11, Lanes 4, 6, 8-14) derived from two different crosses involving W1. The SC Westar line (Figure 11, Lane 1) and SC progeny (Figure 11, Lanes 5, 7 and 15-19) from the crosses did not contain these fragments.

To isolate the W1 associated fragments, oligomers for PCR amplification were designed to highly conserved regions in published SLG sequences as illustrated in Figure 10. The SI-2 (+)-strand primer (SEQ ID NO: 3) corresponds to nucleotides 461-481 of the conserved region of the A14 cDNA and SI-1 (-) strand primer (SEQ ID NO: 4) corresponds to a sequence complimentary to nucleotides 1290-1270 of the conserved region of the A14 cDNA. PCR amplification was performed according to a modification of the method described by Saiki et al. (Science 230:1350, 1985). Two different sources of DNA were used; the W1 homozygote (Figure 11, Lane 2) and the 1581 plant (Figure 11, Lane 4). W1 and 1581 genomic DNA were digested with Hind III and fractionated on a 0.7 % agarose gel. The regions in the gel spanning 3.6 to 3.9kb and 6.5 to 6.9kb were excised and the DNA was isolated by electroelution. Approximately 50ng of the fractionated genomic DNA was used in a 100 µl PCR reaction with 1µM of each primer, SI-1 (SEQ ID No. 4) and SI-2 (SEQ ID No. 3), 200µM each dNTP, and 2.5 units of Taq polymeras . The PCR conditions were 94°C for 1.5 min., 45°C for 1 min., and 72°C for 1.5 min. for a total

of 30 cycles. The PCR products were cloned into pBluescript (Stratagene, LaJolla, CA) by standard methods. The expected product size was roughly 800 bp starting approximately 400 bp from the 5' end. Using dideoxy sequencing and the Sequenase enzyme (United States Biochemicals), the cloned PCR products were partially sequenced (as described above, and in Goring et al. (1992a)) to determine their identity, and then used as probes on genomic blots. From the 6.5kb region, two different clones were obtained. One clone was specific for the 1581 plant. The second clone, 910, hybridized to the upper W1 specific band (Figure 11).

From the 3.6kb region, only one PCR clone, 1631, having about 800 bp was obtained and it was found to hybridize to the lower W1 specific band (Figure 3). (An RNA blot analysis, which was performed (not shown), revealed that only a single gene was highly expressed in the stigma. That single gene was further characterized as described below.) The sequence analysis of the 800 bp genomic PCR clone (Figure 1B) showed high levels of homology (89%) to the SLG-910 gene. Notwithstanding the high degree of homology, we produced three specific primers from this 800 bp region that were designed to isolate the remainder of the coding region for the novel gene (now designated as SRK-910). The three specific primers are referred to herein as "primer 1" (SEQ ID No. 5), "primer 2" (SEQ ID No. 6), and "primer 3" (SEQ ID No. 7). As shown in Figure 12, primer 1 is a (+) strand primer (SEQ ID No. 5) corresponding to nucleotides 820 to 839 of the SRK-910 gene; primer 2 is a (-) strand primer (SEQ ID No. 6) corresponding to a sequence complementary to nucleotides 1002 to 983 of the SRK-910 gene; and primer 3 is a (+) strand (SEQ ID No. 7) corresponding to nucleotides 1256 to 1275 of the SRK-910 gene.

The 5' end of the SRK-910 gene was amplified using the inverse PCR technique (Ochman et al., 1988). *Hind* III digested W1 genomic DNA from the 3.6 kb region was extracted, circularized by ligation, and amplified with

primers 2 and 3 (SEQ ID Nos. 6 and 7 respectively). Primers 2 and 3 were oriented in opposite directions (Figure 1C). Sequence analysis revealed that the inverse PCR fragment contained 59 bp at the 3' end of the SLG homology region plus approximately 400 bp of an intron following this region. At the 5' end, 1 kb of the coding region with no introns, and another 1.8 kb upstream of the initiation codon was present.

The 3' end of the SRK-910 gene was isolated by amplification of pistil cDNA using the RACE procedure (Frohman et al., 1988) with two sequential rounds of amplification utilizing primers 1 and 3 (SEQ ID Nos. 5 and 7 respectively). This PCR cDNA fragment was 1.5 kb in length starting at the 3' end of the SLG homology region (Figure 1D).

The sequence of the SRK-910 coding regions was derived from the three overlapping clones in Figure 1B-D. For the 3' end, three different PCR cDNA clones were sequenced and found to have small insertions or deleti ns which were not present in the other clones. Stein et al. (1991) found that another gene, a *B. oleracea* SRK gene, contained a large intron following the SLG homology region followed by 5 small introns in the remainder of the 3' end. In the present invention, the changes that were observed corresponded to the location of some of these introns, yet each cDNA clone had a different alteration suggesting that the changes were due to splicing errors. Clone 26 contained a 88 bp insert at the site of the 4th intron. Clone 24 had a 5 bp deletion at the 3rd intron splice site. The last clone, clone 10, contained a 41 bp deletion by the 4th intron and a 20 bp insert at the 5th intron. Since the alterations in each of these clones were different, a correct cDNA could be constructed using clones 24 and 26.

To determine if the SRK-910 gene was frequently processed incorrectly or if a cloning problem led to the isolation of altered cDNAs, cDNA PCR products were analyzed before the cloning stage. Using primers outside

of the 5 small introns (nucleotides 1378-2323), stigma cDNA, genomic DNA, and the three altered cDNA clones wer amplified. (Figure 2). For the stigma cDNA PCR products, a clear band was detected, in addition to a faint smear of slightly larger molecular weight products (not shown). The PCR products were digested with *Alu* I which produces 5 small fragments (298, 197, 183, 175, and 91 bp) for the correct cDNA clone (Figure 2, Lane 2). The PCR products from the directly amplified stigma cDNA samples showed the same digest patterns (Figure 2, Lanes 3 and 4) as the correct cDNA clone. The PCR products from the altered cDNA clones show some differences (Figure 2, Lanes 5-6, marked by dots). The insertion in clone 26 contained two *Alu* I sites producing two small bands (47 and 30 bp; Figure 2, Lane 7) which are also present in the genomic sample (Figure 2, Lane 1) confirming that the insert originates from the gene. Thus, the majority of the SRK-910 message is processed correctly.

Segregation of the SRK-910 Gene With Self-Incompatibility in the W1 Line

During the initial analysis of the W1 S-locus, it became apparent that there were other related genes in the W1 genome, such as non-functional S-loci present in the original self-compatible Westar line, and distinct loci which share homology to the S-locus (see Lalonde et al., 1989; Boyes et al., 1991; and Goring et al., 1992a). Thus, it was important to confirm that the isolated SRK-910 gene is associated with W1 self-incompatibility. A segregating F_2 population was produced by crossing a homozygous self-compatible Westar plant. The heterozygous F_1, plants were self-pollinated to produce a F_2 population of W1/W1, W1/Westar, and Westar/Westar plants. These plants were then tested for self-incompatibility by self-pollination, and reciprocal crosses to the W1 and Westar parental lines (Goring et al. (1992a)). In addition, genomic DNA samples from these plants were hybridized to the 2.8 kb SRK-910 coding

region to determine if this gene segregated with W1 self-incompatibility (Figure 3).

TABLE I

ABBREVIATIONS FOR AMINO ACIDS

Amino Acid	Three-Letter Abbreviations	One-Letter Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic Acid	Asp	D
Asparagine or aspartic acid	Asx	B
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic Acid	Glu	E
Glutamine or glutamic acid	Glx	Z
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

The SRK-910 clone was found to hybridize to two *Hind* III fragments only in DNA samples extracted from plants displaying the W1 self-incompatibility phenotype (Figur 3, Lanes 1, 2 and 4-11). Accordingly, the SRK-910 gene represents a second gene at the W1 S-locus that segregates with self-incompatibility. (In Goring et al. (1992a), we established that the SLG-910 gene at the W1 S-locus also segregated with self-incompatibility.)

Sequence Analysis of the SRK-910 Gene

The SRK-910 DNA sequence has an open reading frame of 2574 bp for a predicted protein sequence of 858 amino acids, followed by a small 3' untranslated region represented by nucleotides 2585 to 2749 (Figure 4). Nucleotides 1 to 1315 of Figure 4 represent the portion of the SRK-910 gene which cross-hybridized to the SLG-A14 probe used in the initial study. There are features in this sequence that are representative of SLG alleles such as the 12 cysteine residues conserved in all SLG sequences (Figure 4, dashed line above). In addition, there are seven potential N-glycosylation sites (Figur 4, bold-italics) in keeping with the fact that the SLG proteins are glycosylated (Takayama et al., 1986, 1989). A hydropathy plot (Figure 5) of the predicted amino acid sequence shows a signal peptide at the N-terminal end and a transmembrane domain separating the SLG homologous N-terminus with the rest of the coding region (Figure 4, underlined; Figure 5). Homology comparisons (Figure 5) of the SRK-910 SLG domain to other SLG alleles indicated that the SRK-910 allele is most closely related to its SLG counterpart at the same locus, the SLG-910 allele. At the DNA level, there is 89.9% homology between the two genes and 84.1% similarity at the amino acid level (Figure 5). Amino acid homologies to other phenotypically strong SLG alleles range from 72% to 79% (not shown).

Th predicted amino acid sequence of the 3' end of the gene, after the transmembrane domain, contains

conserved amino acids found in serine/threonine protein kinases (Hanks et al., 1988). In plants, there have been three other reports of receptor kinases and all have contained the serine/threonine protein kinase consensus sequences (Walker & Zheng, 1990; Stein et al., 1991; Tobias et al., 1992). Alignment of the SRK-910 sequence to these other receptor kinases show that is most similar to the SRK-gene isolated from *B. oleracea* (Figure 5). Since comparisons of SLG alleles from *B. oleracea* and *B. campestris* have shown that these alleles are equally similar across species as they are within species (Dwyer et al., 1991; Goring et al., 1992a), the high level of similarity between SRK-910 *(B. campestris* origin) and SRK-6 (*B. oleracea* origin) is not surprising. However, a comparison between these two genes of the SLG domain and kinase domain separately shows an interesting feature. In the kinase domain, the homology between the SRK-910 and SRK-6 DNA sequences is 89.6% and the amino acid similarity is 84.1% with a difference of 5.5%. In the receptor domain, the DNA homology is 84.8%; however, the amino acid similarity decreases by 9.4% to 75.4%. Since it is likely that the extracellular receptor domain determines the specificity of each allele, there appears to have been a greater selection for base substitutions in this region which alter the amino acid sequence. There is a significant, but lower level of homology to the *B. oleracea* pollen recessive SRK-2 gene and the *Arabidopsis* ARK-1 gene. The ARK-1 gene is not a S-locus gene because *Arabidopsis*, despite being closely related to the *Brassica* family, does not possess a self-incompatibility system. The corn ZMPK-1 gene is most distantly related to the SRK-910 gene with higher levels of homology detected in the kinase domain (Figure 5).

Hanks et al. (1988) have shown in an alignment of other eucaryotic protein kinases that within 11 domains, there are several absolutely conserved amino acids and several cons rved amino acid groups. An alignment of the eleven domains within the kinase region of the five plant

receptor kinases is shown in Figure 6 with the consensus amino acids indicated on the top line. All of the absolutely conserved amino acids (in bold) are present. In addition, the conserved amino acid groups (regular type) are also present. The two underlined regions represent consensus sequences differentiating between serine/threonine kinases and tyrosine kinases. While the sequence of the corn ZMPK-1 protein most closely represents the two consensus regions, the SRK-910 is most divergent, especially in the first consensus region (Figure 6). The second consensus region in the SRK-910 is closer to the serine/threonine kinase consensus sequence than that found for tyrosine kinases (P-I/V-K/R-W-T/M-A-P-E). Recently, a number of protein kinases have been isolated which contain the consensus serine/threonine sequences, but demonstrate serine/threonine and tyrosine (STY) activity when tested. Seger et al. (1991) noted some sequence homologi s specific to these STY kinases in domain XI. A search for these consensus sequences in domain XI of the plant kinases did not reveal any similarities.

Kinase Activity of The SRK-910 Protein

To confirm that the SRK-910 is an active kinase and to determine the specificity of the kinase activity, fusion proteins were synthesized in *E. coli* and assayed for kinase activity. The kinase domain (nucleotides 1383-2749) was placed in pGEX-3X (Smith & Johnson, 1988) which creates a protein fusion between glutathione S-transferase (GST) and the SRK-910 kinase, and in pAGEX-2T (Smith & Wildeman, in preparation) which contains two IgG binding domains from *S. aureas* protein A in front of the GST protein. These two constructs produce fusion proteins of 72 kD and 83 kD in size, respectively. Purified fusion proteins were assayed for kinase activity based on autophosphorylation in the presence of γ^{32}P-ATP. To demonstrate that phosphorylation of the fusion proteins was not the result of bacterial kinase activity, a mutant SRK-910 protein

("kinase") was also constructed by substituting an alanine residue for the invariant lysine in domain II (Figure 6). The mutant SRK-910 protein lacked kinase activity.

A coomassie blue stain of the protein gel showed that both wild type and mutant fusion proteins of the expected sizes could be detected (Figure 7A, Lanes 4-7, marked by dots), and were not present in the control lanes of HB101 (Figure 7A, Lane 1), pGEX-3X (Figure 7A, Lane 2), and pAGEX-2T (Figure 7A, Lane 3). The smaller proteins in Lanes 4-7 are either *E. coli* proteins carried through the purification, or degradation products from the fusion proteins. An autoradiogram of the protein gel showed that only the wild-type fusion proteins were labeled with ^{32}P (Figure 7B, Lanes 4 and 6, marked by dots). Thus, the SRK-910 gene does contain an active kinase, and mutation of the invariant lysine to alanine resulted in loss of activity. To determine the amino acid specificity of the SRK-910 kinase, the phosphorylated fusion proteins were extracted from the protein gel and subjected to phosphoamino acid analysis. For the AGST-kinase fusion protein (83 kD), only serine and threonine residues were phosphorylated (Figure 7C). Similar results were also seen for the GEX-kinase protein (72 kD, not shown). Phosphorylation of tyrosine residues could not be detected even after a long exposure of the autoradiogram (not shown). Thus, the SRK-910 protein encodes a serine/threonine kinase.

Expression Of The SRK-910 Gene

Poly A+RNA samples extracted from various tissu s were subjected to RNA blot analysis to determine the expression patterns of the SRK-910 gene. The results showed that SRK-910 mRNA transcripts were present predominantly in the pistil at all three stages sampled. (Figure 8A, Lanes 6-8, marked by arrow). This is a similar pattern of expression to the SLG-910 gene (Goring et al., 1992a). However, the SRK-910 transcripts are present at considerably lower levels in comparisons to

the SLG-910 transcripts (not shown). As a result of the sequence similarity between the SRK-910 and SLG-910 genes, and the high abundance of the SLG-910 messag , some cross hybridization was detected in the RNA blot analysis as seen by the presence of the lower band (Figure 8A, Lanes 6-8). Stein et al. (1991) also found that the *B. oleracea* SRK-6 gene was expressed at low levels in the anther tissue.

We investigated the expression of the SRK-910 gene in the same tissues using a more sensitive PCR assay. First strand cDNA synthesized from total RNA was amplified with two SRK-910 specific primers, primer 1 (nucleotides 1256-1273; SEQ ID No. 5) and primer 4 (th (-) strand for nucleotides 2304-2323; SEQ ID No. 8) that span the kinase region which contains several introns. After 25 cycles, SRK-910 PCR products were only detected in the stigma samples with ethidium bromide staining (Figure 8B). However, DNA blot analysis of the PCR samples also revealed PCR products hybridizing to the SRK-910 probe in the anther samples, but at a much lower level than seen for the stigma samples (Figure 8C). Hybridizing PCR Products were not present in the petal and leaf samples. Thus, there is also weak expression of the SRK-910 gene in the anther.

The amino acid sequences of the receptor domain of the SRK and of the SLG presumably are crucial for differentiating between allele-specific ligand molecules synthesized in the tapetum of the male parent and present in the exine of the pollen. The predicted amino acid sequence of the SLG-910 gene shows high levels of homology to the receptor portion of the SRK-910 protein. At the amino acid level, the SRK-910 and SLG-910 proteins share 84% homology. If these two proteins are able to bind the same ligand specific to the W1 S-locus, some shared sequences unique to only these two proteins would be expected. Alignment of several SLG alleles has shown domains of conserved and variable regions (Dwyer et al., 1991). Since the variable regions are likely to be

responsible for the specificity of each allele, these regions were examined for conserved amino acids between the SLG-910 and SRK-910 sequences, but obvious conserved stretches were not observed. However, single amino acids which would be brought together when the protein is folded correctly would not be easily detected.

The carboxy-half of the SRK-910 protein was found to phosphorylate only serine and threonine residues and did not appear to phosphorylate tyrosine residues as demonstrated for STY protein kinases. When the kinase domains from the plant receptor kinases were aligned, in addition to the serine/threonine consensus sequences, they contained all of the conserved amino acids that have been found in protein kinases isolated from other eucaryotes. Some of these conserved amino acids hav been implicated in ATP binding or proton transfer, and thus are important for the enzyme activity (Hanks et al., 1988). In the case of the invariant lysine in domain II, we have demonstrated that altering this amino acid will also abolish kinase activity in the SRK-910 protein.

SLG proteins have been found outside of the cell membrane and localized to the cell wall of the stigma papillae cells (Kandasamy et al., 1989). In the present invention, the structure of the SRK predicted protein sequence indicates that it is localized in the cell membrane. This type of truncated secreted receptor and transmembrane receptor combination has been detected in other systems. However, in these other examples, the truncated receptor has been generated by alternate splicing of the same gene producing the transmembrane receptor and consequently, the two protein products are identical or nearly identical in sequence (Johnson et al., 1990; Petch et al., 1990). The precise role of these truncated receptors in signal transduction is not known. In one example, there is a differential expression of the truncated and full length receptors leading to th proposition that the truncated receptors

may represent another level of regulation to modulate ligand responsiveness by the transmembrane receptor (Petch et al., 1990). In the case of the growth hormone receptor, the truncated receptor represents the growth hormone serum binding protein (Leung et al., 1987). Since plants also have a thick cell wall surrounding th cell membrane, the S-locus glycoproteins (SLG) may serv to recruit ligand molecules for the S-locus serine/threonine receptor kinases.

Unless signal transduction occurs through interactions between the allele specific SLG and SRK proteins, a third protein, the ligand which activates the receptor kinase must be required. The highly localized self-incompatibility response suggests that its expression would be anther specific and would have evolved co-linearly with the SLG and SRK genes at the S-locus.

While the immediate downstream targets of the activated receptor serine/threonine kinase are not known, one of the rapid responses that has been clearly documented is the deposition of (1,3)-ß-glucan (callose) in the stigma papillae cell in contact with the self-incompatible pollen (Heslop-Harrison et al., 1974).

Introduction Of The Isolated cDNAs For The SRK-910 And SLG-910 Alleles Into Plants, Plants Cells And/Or Plant Protoplasts

Both the SRK-910 allele and the SLG-910 allele (Goring et al., 1992a) have been shown to segregate with the SI-phenotype in *Brassica*. Additionally, neither gene appears to be present in self-compatible plants. Both genes show a tissue specific expression pattern in SI plants which corresponds to the tissues responsible for self-incompatibility in *Brassica*. The specific association between these genes and their expression with the SI phenotype in plants, clearly establishes the importance of these genes in the self-incompatibility mechanism of *Brassica*.

On this basis, the present invention also relates to a transfer vector comprising the isolated cDNA of the SRK-allele (SEQ ID No. 1) which is useful in the transformation of SC plants, plant cells from SC plants and/or protoplasts from SC plants which are capable of expressing the SI phenotype. Preferably, the transf r vector includes the isolated cDNA from two alleles that are associated with self-incompatibility, i.e., the cDNA for the SLG-910 allele, which is disclosed in Goring et al. (1992a), and the cDNA for the SRK-910 allele (SEQ ID No. 1), which is taught herein.

The vector of the present invention may be introduced into SC plants, plant cells and/or plant protoplasts by standard methodologies including but not limited to calcium-phosphate co-precipitation techniques, protoplast fusion, electroporation, microprojectile mediated transfer, by infection with bacteria (e.g., *Agrobacterium tumifaciens)*, viruses or other infectious agents capable of delivering nucleic acids to recipient plants, plant cells and/or plant protoplasts capable of expressing the SI genes and the SI phenotype.

By way of example, the bacteria *Agrobacterium tumifaciens* may be used to introduce the vectors of the present invention into SC plants, plant cells and/or plant protoplasts. More specifically, the isolated SRK-910 (SEQ ID No. 1) and SLG-910 (Goring et al., 1992a) cDNAs may be cloned into the Ti plasmid pBI101.2 by standard cloning procedures. The chimeric plasmid comprising pBI101.2 and the cDNAs for SRK-910 and SLG-910 may be introduced into *Agrobacterium tumifaciens* LBA4404 (Oomstal, Gene 14: 33-50, 1981) by standard transformation techniques well known in the art. (Horsh et al., Science 277:1229-1231, 1985; Arnoldo, M., et al., Genome [in press]). The resulting *Agrobacterium tumifaciens* may then be used to introduce the SI cDNA into SC plants, plant tissues or plant protoplasts of *Brassica* by standard infection procedures.

It is contemplated that the introduction of a transfer vector carrying the cDNAs for both the SRK-910 and the SLG-910 alleles, such as those described above, into SC plants, plant cells and/or plant protoplasts will result in the expression of the SI phenotype in plants which were previously self-compatible.

Method For The Rapid Screening of *Brassica* Seedlings For the Presence Of The SRK-910 Allele

In order to screen *Brassica* seedlings for the presence of a particular SI allele, the plants b ing tested are typically grown to flowering and then crossed to tester plant lines carrying known alleles as described above. This process is both time-consuming and expensive. In order to overcome these problems, the present invention also relates to a method for the rapid screening of *Brassica* seedlings for the presence of SRK-910 allele. The method employs the polymerase chain reaction to amplify the genomic DNA obtained from the *Brassica* seedling of interest. To have specificity for the SRK-910 allele, the method utilizes oligonucleotide probes selected from unique regions of the SRK-910 allele. Suitable nucleotide probes for detecting the presence of the allele are primers 1, 2, 3, or 4 as taught herein. In particular, the method for screening a *Brassica* seedling for the SRK-910 allele comprises th steps of:

a) obtaining genomic DNA from the tissue of a *Brassica* seedling suspected of having the SRK-910 allele;

b) combining the genomic DNA with a (+) strand oligonucleotide and a (-) strand oligonucleotide that are both SRK-910 specific and capable of priming the amplification of the SRK-910 allele, the oligonucleotides comprising:

i. a (+) strand oligonucleotide having the sequence TCCGGAATTACTTTGATGAC (SEQ ID No. 7), and a (-) strand oligonucleotide having the sequence GAAAGGTTGCTGGTAATGAT (SEQ ID No. 8); or

ii. a (+) strand oligonucleotide having the sequence AGTAACGATGAGTATTTGGC (SEQ ID No. 5), and a (-) strand oligonucleotide having either the sequence CATATTGAAGGGCTTGAAAC (SEQ ID No. 6) or the sequence GAAAGGTTGCTGGTAATGAT (SEQ ID No. 8);

c) amplifying the allele using the polymerase chain reaction to render the allele detectable; and

d) determining the presence of the SRK-910 allele by detecting the PCR amplification products that are specific for the SRK-910 allele.

In this method, genomic DNA is prepared from seedlings by the method of Edwards and Thompson, (Nucl. Acids. Res. 19:1349, 1991). Genomic DNA is then amplified in a polymerase chain reaction using a pair of specific primers that are preferably oriented in opposite directions. The step of determining the presence of the allele, via its amplification products, may be accomplished by any of the standard detection techniques already described herein. It is also within the scope of the present invention to label the SRK-910 probe or a specific oligonucleotide, such as those recited in Step (b), for use in detecting the PCR amplification products. The use of radioactive labels, such as ^{32}P, for the labeling of nucleotide probes is well known in the art.

Because the SRK-910 gene and self-incompatibility segregate together, the present invention is further directed to screening a *Brassica* seedling for self-incompatibility comprising the steps of:

a) obtaining genomic DNA from the tissue of a *Brassica* seedling suspected of having the self-incompatibility phenotype;

b) combining the genomic DNA with a (+) strand oligonucleotide and a (-) strand oligonucleotide that are both SRK-910 specific and capable of priming the amplification of the SRK-910 allele, the oligonucleotides comprising:

i. a (+) strand oligonucleotide having th sequence TCCGGAATTACTTTGATGAC (SEQ ID No. 7), and a (-) strand oligonucleotide having the sequence GAAAGGTTGCTGGTAATGAT (SEQ ID No. 8); or

ii. a (+) strand oligonucleotide having the sequence AGTAACGATGAGTATTTGGC (SEQ ID No. 5), and a (-) strand oligonucleotide having either the sequence CATATTGAAGGGCTTGAAAC (SEQ ID No. 6) or th sequence GAAAGGTTGCTGGTAATGAT (SEQ ID No. 8);

c) amplifying the SRK-910 allele, which is associated with the self-incompatibility phenotype, using the polymerase chain reaction (PCR) technique to render the allele detectable; and

d) determining the presence of the self-incompatibility phenotype by detecting the presence of the PCR amplification products that are specific for the SRK-910 allele.

EXPERIMENTAL PROCEDURES

1. Cloning of The SRK-910 Gene

PCR amplification of the 800bp internal genomic fragment has been described above and in Goring et al. (1992a). For the 3' RACE procedure, we utilized the cDNA synthesis, the dT_{17}-Adaptor (SEQ ID No. 9) and Adaptor (SEQ ID No. 10) primers of Figure 10, and the PCR amplification as described in Goring et al. (1992a),

except that approximately 400 ng of poly A+ RNA was used for the cDNA synthesis. After the first round of amplification with the SRK-910 specific primer, primer 1 (SEQ ID No. 5), a specific band was not detected. The resulting products (faint smears) were fractionated on a 1% low melting-point agarose gel and agarose plugs were removed with pasteur pipettes (Zintz & Beebe, 1991) in the range of 1.5 to 5 kb. The DNA-containing agarose plugs were melted at 70°C for 10 minutes and subjected to a second round of PCR amplification using 200 nM each of the Adaptor (SEQ ID No. 10) and SRK-910 specific primer, primer 3 (SEQ ID No. 7) for 30 cycles.

For the inverse PCR, 100 ng of size fractionated (3.6 to 3.9 kb), *Hind* III digested, Wl genomic DNA was ligated under dilute conditions promoting circularization (Ochman et al., 1988). After 40 cycles, the PCR reaction was precipitated with ethanol and size fractionated on a 1 w melting point agarose gel. A faint band could be detected at approximately 3.5 kb in size, and agaros plugs were removed as described above and amplified for 21 cycles. All PCR products were cloned into pBluescript (Stratagene, LaJolla, CA.) and sequenced as described herein. Two to three different clones from separate PCR reactions were sequenced for each section to solve any discrepancies in the SRK-910 sequence resulting from Taq polymerase errors. DNA and protein sequence analysis was carried out using the DNASIS and PROSIS software (Pharmacia, Piscataway, NJ).

2. Intron Analysis

First strand cDNA primed with primer 4 (i.e., nucleotides complimentary to 2304-2323; SEQ ID No. 8), the 3' RACE cDNA clones, and a WI genomic DNA sample were amplified with two primers (20 bp each) encompassing nucleotides 1378 to 2323 of the SRK-910 gene. Th resulting PCR products were gel-purified from low molecular weight PCR products and digested with *Alu* I. The digested samples were labelled with ^{35}S-dATP by an exchange reaction with the Klenow polymerase fragment

(Sambrook et al., 1989), and size fractionated on a 5% polyacrylamide gel. The gel was then dried and exposed to X-ray film.

3. Fusion Proteins and Kinase Assays

Mutation of the invariant lysine to alanine was carried using PCR mutagenesis. Two overlapping regions (nucleotides 1256-1681; and nucleotides 1378-1779) w re amplified with one of the inside primers (nucleotides 1660-1681) introducing the AAAGCA change. The two separate PCR fragments (approximately 400 bp in length) were mixed together and reamplified with the outside primers (nucleotides 1256-1779) to produce a 523 bp fragment which was then cloned and sequenced. With this strategy, half of the clones carried the introduced mutation. A 400 bp *Bcl I/Eco* RI fragment (nucleotides 1383-1761) containing the mutation was then cloned into the kinase domain to replace the wild type sequence. The GST fusions were made using the 3' end of the clon starting at the *Bcl* I site which occurs near the end of the transmembrane domain. The 5' end (*Bcl* I) was placed in frame to the *Sma* I site in a pAGET-2T (Smith & Wildeman, in preparation).

For the kinase assays, 50 ml HB101 cultures carrying the various fusion constructs were grown at 37°C to an OD_{600} of 0.6 (faster growing cultures were diluted during growth). IPTG was then added to a final concentration of 1mM and the cultures were incubated at 37°C for one hour. Purification of the fusion proteins on glutathion agarose beads was carried out essentially as described in Smith & Johnson (1988), except that instead of PBS, the extraction buffer of Douville et al (1992) was used for resuspension and washes. In addition, the protein extracts were mixed with the glutathione agarose beads for 30 minutes at room temperature. Following the washes, the agarose beads containing the fusion proteins were washed an additional two times with the kinase buffer (30mM Tris pH 7.5, 20mM HEPES pH 7.1, 10mM $MgCl_2$, 2mM $MnCl_2$; Douville et al. 1992) and resuspended in a

final volume of 50 μl kinase buffer. 25 μCi of γ^{32}-P-ATP (6000 Ci/mmol) was added to each sample and left at room temperature for 30 minutes. The beads were spun down, resuspended in 20 μl of 2 X sample buffer, boiled for 5 minutes and electrophoresed through an 8.5% SDS-PAGE gel. Subsequently, the SDS-PAGE gel was stained with coomassie blue, dried down and exposed overnight to X-ray film at -70°C. The fusion proteins which could be detected by the coomassie blue stain were excised and extracted from the gel, and subjected to phosphoamino acid analysis as described in Cooper et al. (1983) and Boyle et al. (1991).

4. RNA and DNA Blot Analysis, And PCR Expression Analysis

The poly A+RNA samples for the RNA blot analysis were extracted using the Micro-FastTract mRNA isolation kits (Invitrogen). Gel electrophoresis and blot hybridization were performed using standard techniques (for example, see Goring et al., 1992a). Following hybridization, th blots were washed twice in 0.1X SSC and 0.1% SDS for 30 minutes. The washing temperatures were 67°C for the SRK-910 probe and 50°C for the *Arabidopsis* actin probe.

To examine the expression of SRK-910 gene using PCR, total RNA samples were extracted using the method of Jones et al., (1985). Ten micrograms of total RNA was used for first strand cDNA synthesis using random hexamers and the procedure of Harvey and Darlison (1991). Three PCR reactions were set up from each batch of cDNA, and allowed to amplify for 25, 35, and 45 cycles, respectively. One-quarter of the PCR reaction was subjected to gel electrophoresis. The PCR products were visualized with ethidium bromide staining and then subjected to DNA blot analysis.

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DEPOSITS

Brassica napus line W1 seeds were deposited with th American Type Culture Collection (12301 Parklawn Drive, Rockville, MD 20852, U.S.A.) on October 7, 1993.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Rothstein, Steven J.
Goring, Daphne

(ii) TITLE OF INVENTION: S-LOCUS RECEPTOR KINASE GENE IN A SELF-INCOMPATIBLE BRASSICA NAPUS LINE

(iii) NUMBER OF SEQUENCES: 10

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Foley & Lardner
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(C) CITY: Washington
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/959,945
(B) FILING DATE: 08 OCTOBER 1992
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Bent, Stephen A.
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(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 202-672-5300
(B) TELEFAX: 202-672-5399

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2749 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Brassica napus

(B) STRAIN: oleifera
(C) INDIVIDUAL ISOLATE: W1

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT: S-locus

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..2574

(x) PUBLICATION INFORMATION:
(A) AUTHORS: ROTHSTEIN, STEVEN J.
GORING, DAPHNE
(B) TITLE: THE S-LOCUS RECEPTOR KINASE GENE IN A SELF-INCOMPATIBLE BRASSICA NAPUS LINE
(K) RELEVANT RESIDUES IN SEQ ID NO:1: FROM 1 TO 2749

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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 1               5                  10                  15

TTG CTC GTC TTT TTC GTC ATG TTT CTA TTT CAT CCT GCC CTT TCG ATC        96
Leu Leu Val Phe Phe Val Met Phe Leu Phe His Pro Ala Leu Ser Ile
            20                  25                  30

CAT ATC AAC ACT TTG TCG TCT ACA GAA TCT CTT ACA ATC TCA AAC AAC       144
His Ile Asn Thr Leu Ser Ser Thr Glu Ser Leu Thr Ile Ser Asn Asn
        35                  40                  45

AGA ACA CTT GTG TCT CCA GGT AAT GTC TTC GAG CTC GGC TTC TTT AGA       192
Arg Thr Leu Val Ser Pro Gly Asn Val Phe Glu Leu Gly Phe Phe Arg
    50                  55                  60

ACC ACC TCA AGT TCT CGT TGG TAT CTC GGG ATA TGG TAC AAG AAT TTG       240
Thr Thr Ser Ser Ser Arg Trp Tyr Leu Gly Ile Trp Tyr Lys Asn Leu
65                  70                  75                  80

CCC TAT AAA ACC TAT GTT TGG GTT GCA AAC AGA GAT AAC CCT CTC TCC       288
Pro Tyr Lys Thr Tyr Val Trp Val Ala Asn Arg Asp Asn Pro Leu Ser
                85                  90                  95

GAT TCC ATT GGT ACG CTC AAA ATC TCC AAC ATG AAC CTT GTC CTC CTC       336
Asp Ser Ile Gly Thr Leu Lys Ile Ser Asn Met Asn Leu Val Leu Leu
            100                 105                 110

GAC CAC TCT AAT AAA TCT GTT TGG TCG ACG AAT CTG ACT AGA GGA AAT       384
Asp His Ser Asn Lys Ser Val Trp Ser Thr Asn Leu Thr Arg Gly Asn
        115                 120                 125

GAG AGA TCT CCG GTG GTG GCA GAG CTT CTG GAG AAC GGA AAC TTC GTC       432
Glu Arg Ser Pro Val Val Ala Glu Leu Leu Glu Asn Gly Asn Phe Val
    130                 135                 140

ATT CGA TAC TCC AAT AAC AAC AAC GCA AGT GGA TTC TTG TGG CAA AGT       480
Ile Arg Tyr Ser Asn Asn Asn Asn Ala Ser Gly Phe Leu Trp Gln Ser
145                 150                 155                 160

TTC GAT TTC CCT ACA GAT ACT TTG CTT CCA GAG ATG AAA CTA GGC TAC       528
Phe Asp Phe Pro Thr Asp Thr Leu Leu Pro Glu Met Lys Leu Gly Tyr
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                    165                 170                 175

GAC CGC AAA AAA GGG CTG AAC AGA TTC CTT ACA GCA TGG AGA AAT TCA      576
Asp Arg Lys Lys Gly Leu Asn Arg Phe Leu Thr Ala Trp Arg Asn Ser
            180                 185                 190

GAT GAT CCC TCA AGC GGG GAA ATC TCG TAC CAA CTA GAC ACT CAA AGA      624
Asp Asp Pro Ser Ser Gly Glu Ile Ser Tyr Gln Leu Asp Thr Gln Arg
        195                 200                 205

GGA ATG CCT GAG TTT TAT CTA TTG AAA AAC GGC GTA CGA GGC TAC CGG      672
Gly Met Pro Glu Phe Tyr Leu Leu Lys Asn Gly Val Arg Gly Tyr Arg
    210                 215                 220

AGT GGT CCA TGG AAT GGA GTC CGA TTT AAT GGC ATA CCA GAG GAC CAA      720
Ser Gly Pro Trp Asn Gly Val Arg Phe Asn Gly Ile Pro Glu Asp Gln
225                 230                 235                 240

AAG TTG AGT TAC ATG GTG TAC AAC TTC ACA GAT AAT AGT GAG GAG GCT      768
Lys Leu Ser Tyr Met Val Tyr Asn Phe Thr Asp Asn Ser Glu Glu Ala
                245                 250                 255

GCT TAT ACA TTT CGA ATG ACC GAC AAG AGC ATC TAC TCG AGA TTG ATA      816
Ala Tyr Thr Phe Arg Met Thr Asp Lys Ser Ile Tyr Ser Arg Leu Ile
            260                 265                 270

ATA AGT AAC GAT GAG TAT TTG GCG CGA CTA ACG TTC ACT CCG ACA TCA      864
Ile Ser Asn Asp Glu Tyr Leu Ala Arg Leu Thr Phe Thr Pro Thr Ser
        275                 280                 285

TGG GAA TGG AAC TTG TTC TGG ACT TCA CCA GAG GAG CCG GAG TGC GAT      912
Trp Glu Trp Asn Leu Phe Trp Thr Ser Pro Glu Glu Pro Glu Cys Asp
    290                 295                 300

GTG TAC AAG ACT TGT GGG TCT TAT GCT TAC TGT GAC GTG AAC ACA TCA      960
Val Tyr Lys Thr Cys Gly Ser Tyr Ala Tyr Cys Asp Val Asn Thr Ser
305                 310                 315                 320

CCA GTG TGT AAC TGT ATC CAA GGT TTC AAG CCC TTC AAT ATG CAG CAG     1008
Pro Val Cys Asn Cys Ile Gln Gly Phe Lys Pro Phe Asn Met Gln Gln
                325                 330                 335

TGG GAA CTG AGA GTC TGG GCA GGT GGG TGT ATA AGG AGG ACG CGG CTT     1056
Trp Glu Leu Arg Val Trp Ala Gly Gly Cys Ile Arg Arg Thr Arg Leu
            340                 345                 350

AGC TGC AAT GGA GAT GGT TTT ACC AGG ATG AAA AAT ATG AAG TTG CCA     1104
Ser Cys Asn Gly Asp Gly Phe Thr Arg Met Lys Asn Met Lys Leu Pro
        355                 360                 365

GAA ACT ACG ATG GCT ATT GTC GAC CGC AGT ATT GGT CGG AAA GAA TGT     1152
Glu Thr Thr Met Ala Ile Val Asp Arg Ser Ile Gly Arg Lys Glu Cys
    370                 375                 380

AAG AAG AGG TGC CTT AGC GAT TGT AAT TGT ACC GCG TTT GCA AAT GCG     1200
Lys Lys Arg Cys Leu Ser Asp Cys Asn Cys Thr Ala Phe Ala Asn Ala
385                 390                 395                 400

GAT ATC CGG AAT GGT GGG TCG GGT TGT GTG ATT TGG ACA GGA GAG CTT     1248
Asp Ile Arg Asn Gly Gly Ser Gly Cys Val Ile Trp Thr Gly Glu Leu
                405                 410                 415
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GAG GAT ATC CGG AAT TAC TTT GAT GAC GGT CAA GAT CTT TAT GTC AGA      1296
Glu Asp Ile Arg Asn Tyr Phe Asp Asp Gly Gln Asp Leu Tyr Val Arg
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TTG GCT GCC GCT GAT CTC GTT AAA AAG AGA AAC GCG AAT GGG AAA ACC      1344
Leu Ala Ala Ala Asp Leu Val Lys Lys Arg Asn Ala Asn Gly Lys Thr
        435                 440                 445

ATA GCG TTG ATT GTT GGA GTT TGT GTT CTG CTT CTT ATG ATC ATG TTC      1392
Ile Ala Leu Ile Val Gly Val Cys Val Leu Leu Leu Met Ile Met Phe
    450                 455                 460

TGC CTC TGG AAA AGG AAA CAA AAG CGA GCA AAA ACA ACT GCA ACA TCT      1440
Cys Leu Trp Lys Arg Lys Gln Lys Arg Ala Lys Thr Thr Ala Thr Ser
465                 470                 475                 480

ATT GTA AAT CGA CAG AGA AAC CAA GAT TTG CTA ATG AAC GGG ATG ATA      1488
Ile Val Asn Arg Gln Arg Asn Gln Asp Leu Leu Met Asn Gly Met Ile
                485                 490                 495

CTA TCA AGC AAG AGA CAG TTG CCT ATA GAG AAC AAA ACT GAG GAA TTG      1536
Leu Ser Ser Lys Arg Gln Leu Pro Ile Glu Asn Lys Thr Glu Glu Leu
            500                 505                 510

GAA CTT CCA TTG ATA GAG TTG GAA GCT GTT GTC AAA GCC ACC GAA AAT      1584
Glu Leu Pro Leu Ile Glu Leu Glu Ala Val Val Lys Ala Thr Glu Asn
        515                 520                 525

TTC TCC AAT TGT AAC AAA CTC GGA CAA GGT GGT TTC GGT ATT GTT TAC      1632
Phe Ser Asn Cys Asn Lys Leu Gly Gln Gly Gly Phe Gly Ile Val Tyr
    530                 535                 540

AAG GGT AGA TTA CTT GAT GGG CAA GAA ATT GCG GTA AAA AGG CTA TCA      1680
Lys Gly Arg Leu Leu Asp Gly Gln Glu Ile Ala Val Lys Arg Leu Ser
545                 550                 555                 560

AAA ACG TCG GTT CAA GGG ACT GGT GAG TTT ATG AAT GAG GTG AGA TTG      1728
Lys Thr Ser Val Gln Gly Thr Gly Glu Phe Met Asn Glu Val Arg Leu
                565                 570                 575

ATC GCG AGG CTT CAG CAT ATA AAC CTT GTC CGA ATT CTT GGC TGT TGC      1776
Ile Ala Arg Leu Gln His Ile Asn Leu Val Arg Ile Leu Gly Cys Cys
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ATT GAG GCA GAC GAG AAG ATG CTG GTA TAT GAG TAT TTA GAA AAT TTA      1824
Ile Glu Ala Asp Glu Lys Met Leu Val Tyr Glu Tyr Leu Glu Asn Leu
        595                 600                 605

AGC CTC GAT TCT TAT CTC TTC GGA AAT AAA CGA AGC TCT ACG TTA AAT      1872
Ser Leu Asp Ser Tyr Leu Phe Gly Asn Lys Arg Ser Ser Thr Leu Asn
    610                 615                 620

TGG AAG GAC AGA TTC AAC ATT ACC AAT GGT GTT GCT CGA GGA CTT TTA      1920
Trp Lys Asp Arg Phe Asn Ile Thr Asn Gly Val Ala Arg Gly Leu Leu
625                 630                 635                 640

TAT CTT CAT CAA GAC TCA CGG TTT AGG ATA ATC CAC AGA GAT ATG AAA      1968
Tyr Leu His Gln Asp Ser Arg Phe Arg Ile Ile His Arg Asp Met Lys
                645                 650                 655

GTA AGT AAC ATT TTG CTT GAT AAA AAT ATG ACA CCA AAG ATC TCG GAT      2016
Val Ser Asn Ile Leu Leu Asp Lys Asn Met Thr Pro Lys Ile Ser Asp
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                660                 665                 670

TTT GGG ATG GCC AGA ATC TTT GCA AGG GAC GAG ACT GAA GCT AAC ACA      2064
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        675                 680                 685

AGG AAG GTG GTC GGA ACT TAC GGC TAC ATG TCT CCG GAG TAC GCA ATG      2112
Arg Lys Val Val Gly Thr Tyr Gly Tyr Met Ser Pro Glu Tyr Ala Met
    690                 695                 700

GAT GGG GTA TTC TCG GAA AAA TCA GAT GTT TTC AGT TTT GGA GTC ATT      2160
Asp Gly Val Phe Ser Glu Lys Ser Asp Val Phe Ser Phe Gly Val Ile
705                 710                 715                 720

GTT CTT GAA ATT GTT AGT GGA AAA AGG AAC AGA GGA TTC TAC AAC TTG      2208
Val Leu Glu Ile Val Ser Gly Lys Arg Asn Arg Gly Phe Tyr Asn Leu
                725                 730                 735

AAC CAC GAA AAC AAT CTT CTA AGC TAT GTA TGG AGT CAC TGG ACG GAG      2256
Asn His Glu Asn Asn Leu Leu Ser Tyr Val Trp Ser His Trp Thr Glu
            740                 745                 750

GGA AGA GCG CTA GAA ATT GTT GAT CCA GTC ATC GTA GAT TCA TTG TCA      2304
Gly Arg Ala Leu Glu Ile Val Asp Pro Val Ile Val Asp Ser Leu Ser
        755                 760                 765

TCA TTA CCA GCA ACC TTT CAA CCA AAA GAA GTT CTA AAA TGC ATA CAA      2352
Ser Leu Pro Ala Thr Phe Gln Pro Lys Glu Val Leu Lys Cys Ile Gln
    770                 775                 780

ATT GGT CTC TTG TGT GTT CAA GAA CGT GCA GAG CAT AGA CCA ACG ATG      2400
Ile Gly Leu Leu Cys Val Gln Glu Arg Ala Glu His Arg Pro Thr Met
785                 790                 795                 800

TCG TCC GTG GTT TGG ATG CTT GGA AGT GAA GCA ACA GAG ATT CCT GAG      2448
Ser Ser Val Val Trp Met Leu Gly Ser Glu Ala Thr Glu Ile Pro Glu
                805                 810                 815

CCT ACA CCG CCA GGT TAT TCC CTC GGA AGA AGT CCT TAT GAA AAT AAT      2496
Pro Thr Pro Pro Gly Tyr Ser Leu Gly Arg Ser Pro Tyr Glu Asn Asn
            820                 825                 830

CCT TCA TCA AGT AGA CAT TGC GAC GAC GAC GAA TCC TGG ACG GTG AAC      2544
Pro Ser Ser Ser Arg His Cys Asp Asp Asp Glu Ser Trp Thr Val Asn
        835                 840                 845

CAG TAC ACC TGC TCA GAC ATC GAT GCC CGG TAGTACGAAA TCCGTTGAGA        2594
Gln Tyr Thr Cys Ser Asp Ile Asp Ala Arg
    850                 855

AAGTTCAGAT AATTAACTAT TGGGGTGACC GGATATTATA AGTGAAAGAA AATAAAATTT    2654

CAATAGTTAA GTTTGTTATT TGATAACCAA ATCTTGTTAT TTCCTGGTGG TGTTGTCATA    2714

TTCGTTTTTC TGAATGAATG TTAAAGTTAT TATTC                               2749
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 858 amino acids
(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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 1               5                  10                  15

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            20                  25                  30

His Ile Asn Thr Leu Ser Ser Thr Glu Ser Leu Thr Ile Ser Asn Asn
        35                  40                  45

Arg Thr Leu Val Ser Pro Gly Asn Val Phe Glu Leu Gly Phe Phe Arg
    50                  55                  60

Thr Thr Ser Ser Ser Arg Trp Tyr Leu Gly Ile Trp Tyr Lys Asn Leu
65                  70                  75                  80

Pro Tyr Lys Thr Tyr Val Trp Val Ala Asn Arg Asp Asn Pro Leu Ser
                85                  90                  95

Asp Ser Ile Gly Thr Leu Lys Ile Ser Asn Met Asn Leu Val Leu Leu
            100                 105                 110

Asp His Ser Asn Lys Ser Val Trp Ser Thr Asn Leu Thr Arg Gly Asn
        115                 120                 125

Glu Arg Ser Pro Val Val Ala Glu Leu Leu Glu Asn Gly Asn Phe Val
    130                 135                 140

Ile Arg Tyr Ser Asn Asn Asn Asn Ala Ser Gly Phe Leu Trp Gln Ser
145                 150                 155                 160

Phe Asp Phe Pro Thr Asp Thr Leu Leu Pro Glu Met Lys Leu Gly Tyr
                165                 170                 175

Asp Arg Lys Lys Gly Leu Asn Arg Phe Leu Thr Ala Trp Arg Asn Ser
            180                 185                 190

Asp Asp Pro Ser Ser Gly Glu Ile Ser Tyr Gln Leu Asp Thr Gln Arg
        195                 200                 205

Gly Met Pro Glu Phe Tyr Leu Leu Lys Asn Gly Val Arg Gly Tyr Arg
    210                 215                 220

Ser Gly Pro Trp Asn Gly Val Arg Phe Asn Gly Ile Pro Glu Asp Gln
225                 230                 235                 240

Lys Leu Ser Tyr Met Val Tyr Asn Phe Thr Asp Asn Ser Glu Glu Ala
                245                 250                 255

Ala Tyr Thr Phe Arg Met Thr Asp Lys Ser Ile Tyr Ser Arg Leu Ile
            260                 265                 270

Ile Ser Asn Asp Glu Tyr Leu Ala Arg Leu Thr Phe Thr Pro Thr Ser
        275                 280                 285

Trp Glu Trp Asn Leu Phe Trp Thr Ser Pro Glu Glu Pro Glu Cys Asp
    290                 295                 300
```

Val Tyr Lys Thr Cys Gly Ser Tyr Ala Tyr Cys Asp Val Asn Thr Ser
305 310 315 320

Pro Val Cys Asn Cys Ile Gln Gly Phe Lys Pro Phe Asn Met Gln Gln
325 330 335

Trp Glu Leu Arg Val Trp Ala Gly Gly Cys Ile Arg Arg Thr Arg Leu
340 345 350

Ser Cys Asn Gly Asp Gly Phe Thr Arg Met Lys Asn Met Lys Leu Pro
355 360 365

Glu Thr Thr Met Ala Ile Val Asp Arg Ser Ile Gly Arg Lys Glu Cys
370 375 380

Lys Lys Arg Cys Leu Ser Asp Cys Asn Cys Thr Ala Phe Ala Asn Ala
385 390 395 400

Asp Ile Arg Asn Gly Gly Ser Gly Cys Val Ile Trp Thr Gly Glu Leu
405 410 415

Glu Asp Ile Arg Asn Tyr Phe Asp Asp Gly Gln Asp Leu Tyr Val Arg
420 425 430

Leu Ala Ala Ala Asp Leu Val Lys Lys Arg Asn Ala Asn Gly Lys Thr
435 440 445

Ile Ala Leu Ile Val Gly Val Cys Val Leu Leu Leu Met Ile Met Phe
450 455 460

Cys Leu Trp Lys Arg Lys Gln Lys Arg Ala Lys Thr Thr Ala Thr Ser
465 470 475 480

Ile Val Asn Arg Gln Arg Asn Gln Asp Leu Leu Met Asn Gly Met Ile
485 490 495

Leu Ser Ser Lys Arg Gln Leu Pro Ile Glu Asn Lys Thr Glu Glu Leu
500 505 510

Glu Leu Pro Leu Ile Glu Leu Glu Ala Val Val Lys Ala Thr Glu Asn
515 520 525

Phe Ser Asn Cys Asn Lys Leu Gly Gln Gly Gly Phe Gly Ile Val Tyr
530 535 540

Lys Gly Arg Leu Leu Asp Gly Gln Glu Ile Ala Val Lys Arg Leu Ser
545 550 555 560

Lys Thr Ser Val Gln Gly Thr Gly Glu Phe Met Asn Glu Val Arg Leu
565 570 575

Ile Ala Arg Leu Gln His Ile Asn Leu Val Arg Ile Leu Gly Cys Cys
580 585 590

Ile Glu Ala Asp Glu Lys Met Leu Val Tyr Glu Tyr Leu Glu Asn Leu
595 600 605

Ser Leu Asp Ser Tyr Leu Phe Gly Asn Lys Arg Ser Ser Thr Leu Asn
610 615 620

Trp Lys Asp Arg Phe Asn Ile Thr Asn Gly Val Ala Arg Gly Leu Leu
625 630 635 640

```
Tyr L u His Gln Asp Ser Arg Phe Arg Ile Ile His Arg Asp Met Lys
                645                 650                 655

Val Ser Asn Ile Leu Leu Asp Lys Asn Met Thr Pro Lys Ile Ser Asp
            660                 665                 670

Phe Gly Met Ala Arg Ile Phe Ala Arg Asp Glu Thr Glu Ala Asn Thr
        675                 680                 685

Arg Lys Val Val Gly Thr Tyr Gly Tyr Met Ser Pro Glu Tyr Ala Met
    690                 695                 700

Asp Gly Val Phe Ser Glu Lys Ser Asp Val Phe Ser Phe Gly Val Ile
705                 710                 715                 720

Val Leu Glu Ile Val Ser Gly Lys Arg Asn Arg Gly Phe Tyr Asn Leu
                725                 730                 735

Asn His Glu Asn Asn Leu Leu Ser Tyr Val Trp Ser His Trp Thr Glu
            740                 745                 750

Gly Arg Ala Leu Glu Ile Val Asp Pro Val Ile Val Asp Ser Leu Ser
        755                 760                 765

Ser Leu Pro Ala Thr Phe Gln Pro Lys Glu Val Leu Lys Cys Ile Gln
    770                 775                 780

Ile Gly Leu Leu Cys Val Gln Glu Arg Ala Glu His Arg Pro Thr Met
785                 790                 795                 800

Ser Ser Val Val Trp Met Leu Gly Ser Glu Ala Thr Glu Ile Pro Glu
                805                 810                 815

Pro Thr Pro Pro Gly Tyr Ser Leu Gly Arg Ser Pro Tyr Glu Asn Asn
            820                 825                 830

Pro Ser Ser Ser Arg His Cys Asp Asp Asp Glu Ser Trp Thr Val Asn
        835                 840                 845

Gln Tyr Thr Cys Ser Asp Ile Asp Ala Arg
    850                 855
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_recomb
(B) LOCATION: 1..26

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTCAAGCTTG TGGCAAAGTT TCGATT 26

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..29

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GTCAAGCTTC TGACATAAAG ATCTTGACC 29

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Brassica napus
(B) STRAIN: oleifera W1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AGTAACGATG AGTATTTGGC 20

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CATATTGAAG GGCTTGAAAC 20

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TCCGGAATTA CTTTGATGAC 20

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GAAAGGTTGC TGGTAATGAT 20

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Brassica napus
(B) STRAIN: oleifera W1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GATCCAGATC TCGAGAAGCT TTTTTTTTTT TTTTTT 36

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..24

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCGGATCCAG ATCTCGAGAA GCTT 24

What Is Claimed Is:

1. An isolated cDNA comprising the nucleotide sequence set forth in Figure 4 (SEQ ID No. 1).

2. The isolated cDNA of Claim 1 consisting essentially of the nucleotide sequence set forth in Figure 4 (SEQ ID No. 1).

3. An isolated gene for a serine/threonine kinase comprising the nucleotide sequence set forth in Figure 4 (SEQ ID No. 1).

4. The isolated gene of Claim 3 consisting essentially of the nucleotide set forth in Figure 4 (SEQ ID No. 1).

5. An isolated DNA sequence encoding for the SRK-910 protein or a kinase active fragment thereof.

6. An isolated DNA sequence encoding for a protein having the amino acid sequence of Figure 9 (SEQ ID No. 2).

7. A DNA probe comprising an oligonucleotide that is capable of hybridizing with the nucleotide sequence of Claim 1 or its complementary sequence, but not with the nucleotide sequences encoding for the partially homologous S-locus glycoproteins.

8. The DNA probe of claim 7 consisting essentially of an oligonucleotide that is a member of the group consisting of:

AGTAACGATGAGTATTTGGC
(SEQ ID No. 5);

CATATTGAAGGGCTTGAAAC
(SEQ ID No. 6);

TCCGGAATTACTTTGATGAC
(SEQ ID No. 7); and

GAAAGGTTGCTGGTAATGAT
(SEQ ID No. 8).

9. A recombinant protein having the amino acid sequence of Figure 9 (SEQ ID No. 2).

10. A method for screening a *Brassica* seedling suspected of having the SRK-910 allele comprising the steps of:

a) obtaining genomic DNA from the tissue of a *Brassica* seedling suspected of having the SRK-910 allele;

b) combining the genomic DNA with a (+) strand oligonucleotide and a (-) strand oligonucleotide that are both SRK-910 specific and capable of acting as primers for the amplification of the SRK-910 allele, said pair of oligonucleotides comprising, either,

i. a (+) strand oligonucleotide having the sequence TCCGGAATTACTTTGATGAC (SEQ ID No. 7), and a (-) strand oligonucleotide having the sequence GAAAGGTTGCTGGTAATGAT (SEQ ID No. 8); or

ii. a (+) strand oligonucleotide having the sequence AGTAACGATGAGTATTTGGC (SEQ ID No. 5), and a (-) strand oligonucleotide having either the sequence CATATTGAAGGGCTTGAAAC (SEQ ID No. 6) or the sequence GAAAGGTTGCTGGTAATGAT (SEQ ID No. 8);

c) amplifying the allele using the polymerase chain reaction to render the allele detectable; and

d) determining the presence of the SRK-910 allele by detecting the PCR amplification products that are specific for the SRK-910 allele.

11. The method of Claim 10 wherein one parent or ancestor of the *Brassica* seedling is in the W1 *Brassica* line.

12. A method for screening a *Brassica* seedling for the presence of the self-incompatibility phenotype, comprising the steps of:

a) obtaining genomic DNA from the tissue of a *Brassica* seedling suspected of having the self-incompatibility phenotype;

b) combining the genomic DNA with a (+) strand oligonucleotide and a (-) strand oligonucleotide that are both SRK-910 specific and capable of acting as primers for the amplification of the SRK-910 allele, said pair of oligonucleotides comprising, either,

i. a (+) strand oligonucleotide having the sequence TCCGGAATTACTTTGATGAC (SEQ ID No. 7), and a (-) strand oligonucleotide having the sequence GAAAGGTTGCTGGTAATGAT (SEQ ID No. 8); or

ii. a (+) strand oligonucleotide having the sequence AGTAACGATGAGTATTTGGC (SEQ ID No. 5), and a (-) strand oligonucleotide having either the sequence CATATTGAAGGGCTTGAAAC (SEQ ID No. 6) or the sequence GAAAGGTTGCTGGTAATGAT (SEQ ID No. 8);

c) amplifying the SRK-910 allele, which is associated with self-incompatibility, using the polymerase chain reaction (PCR) technique to render the allele detectable; and

d) determining the presence of the self-incompatibility phenotype by detecting the presence of the PCR amplification products that are specific for the SRK-910 allele.

13. The method of Claim 12 wherein one parent or ancestor of the *Brassica* seedling is in the W1 *Brassica* line.

14. A vector comprising the isolated cDNA of Claim 1.

15. The vector of Claim 14 further comprising the isolated cDNA for the SLG-910 allele.

16. The vector of Claim 15 further comprising the Ti plasmid.

17. The vector of Claim 16 wherein the Ti plasmid comprises pBI101.2.

18. A method for conferring the self-incompatible phenotype on a self-compatible plant, comprising the step of transferring the vector of Claim 15 into a self-compatible plant, plant tissue or plant protoplastic capable of assimilating said vector and expressing the self-incompatibility phenotype.

19. The method of Claim 18 wherein the self-compatible plant is of the genus *Brassica*.

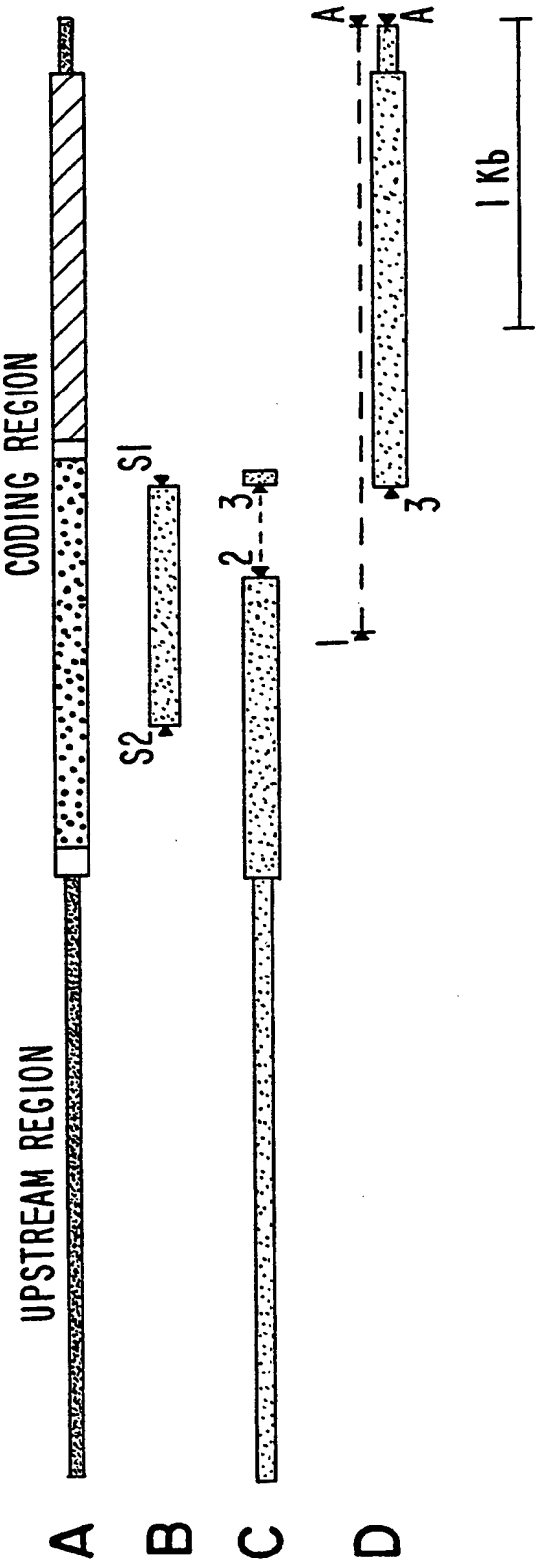

FIG. 1

FIG. 2

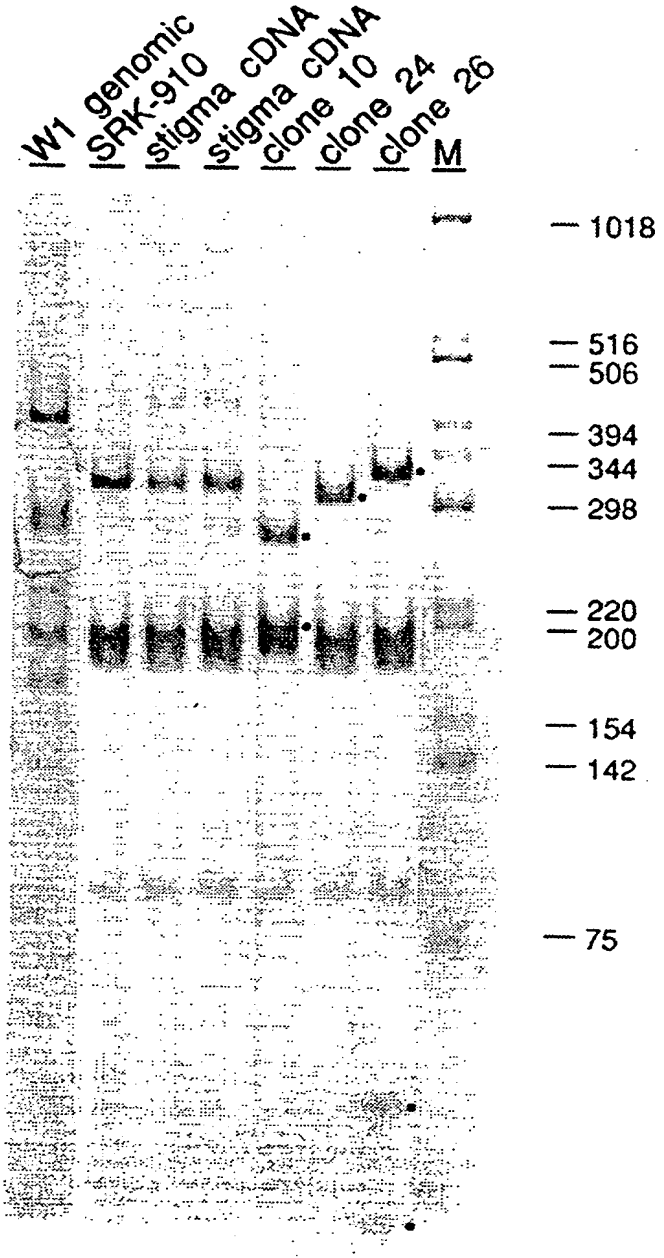

FIG. 3

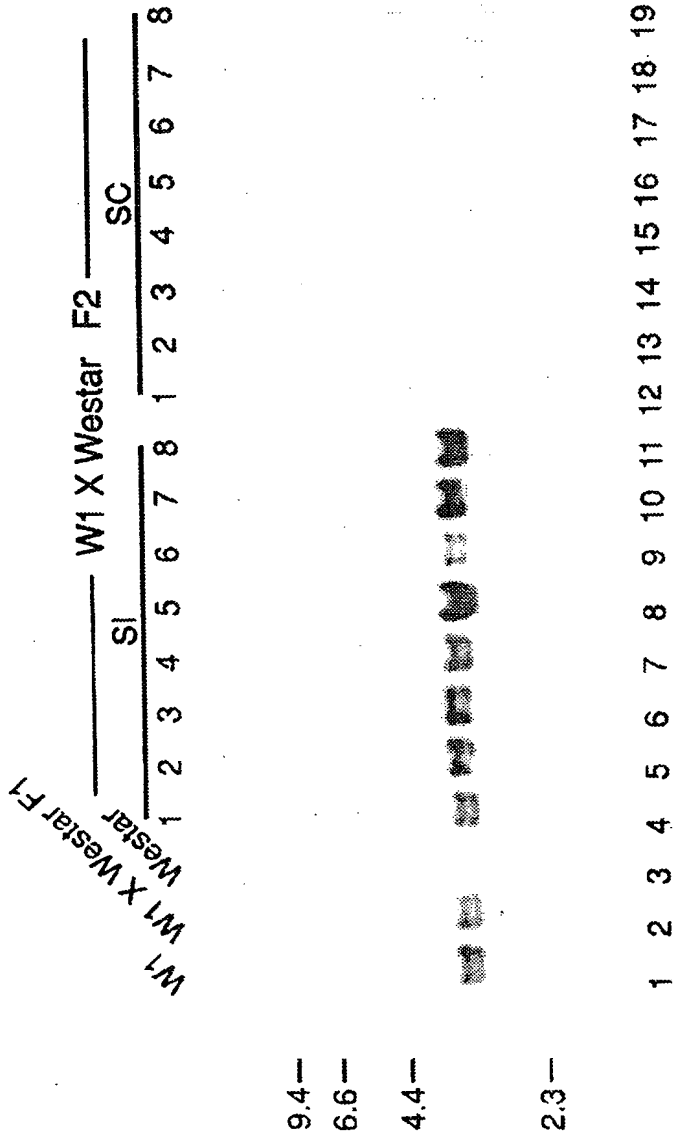

FIG. 4A

```
M  K  G  V  R  K  T  Y  D  S  S  Y  T  L  S  F  L  L  V  F  F  V  M  F  L  F  H  P  A  L  S  I  H  I  N  T  L  S  S  T    40
ATGAAAGGAGTAAGAAAAACCTACGATAGTTCTTACACTTTATCCTTCTTGCTCGTCTTTTTCGTCATGTTTCTATTTCATCCTGCCCTTTCGATCCATATCAACACTTTGTCGTCTACA   120

E  S  L  T  I  S  N  N  R  T  L  V  S  P  G  N  V  F  E  L  G  F  F  R  T  T  S  S  S  R  W  Y  L  G  I  W  Y  K  N  L    80
GAATCTCTTACAATCTCAAACAACAGAACACTTGTGTCTCCAGGTAATGTCTTCGAGCTCGGCTTCTTTAGAACCACCTCAAGTTCTCGTTGGTATCTCGGGATATGGTACAAGAATTTG   240

P  Y  K  T  Y  V  W  V  A  N  R  D  N  P  L  S  D  S  I  G  T  L  K  I  S  N  M  N  L  V  L  L  D  H  S  N  K  S  V  W    120
CCCTATAAAACCTATGTTTGGGTTGCAAACAGAGATAACCCTCTCTCCGATTCCATTGGTACGCTCAAAATCTCCAACATGAACCTTGTCCTCCTCGACCACTCTAATAAATCTGTTTGG   360

S  T  N  L  T  R  G  N  E  R  S  P  V  V  A  E  L  L  E  N  G  N  F  V  I  R  Y  S  N  N  N  N  A  S  G  F  L  W  Q  S    160
TCGACGAATCTGACTAGAGGAAATGAGAGATCTCCGGTGGTGGCAGAGCTTCTGGAGAACGGAAACTTCGTCATTCGATACTCCAATAACAACAACGCAAGTGGATTCTTGTGGCAAAGT   480

F  D  F  P  T  D  T  L  L  P  E  M  K  L  G  Y  D  R  K  K  G  L  N  R  F  L  T  A  W  R  N  S  D  D  P  S  S  G  E  I    200
TTCGATTTCCCTACAGATACTTTGCTTCCAGAGATGAAACTAGGCTACGACCGCAAAAAAGGGCTGAACAGATTCCTTACAGCATGGAGAAATTCAGATGATCCCTCAAGCGGGGAAATC   600

S  Y  Q  L  D  T  Q  R  G  M  P  E  F  Y  L  L  K  N  G  V  R  G  Y  R  S  G  P  W  N  G  V  R  F  N  G  I  P  E  D  Q    240
TCGTACCAACTAGACACTCAAAGAGGAATGCCTGAGTTTTATCTATTGAAAAACGGCGTACGAGGCTACCGGAGTGGTCCATGGAATGGAGTCCGATTTAATGGCATACCAGAGGACCAA   720

K  L  S  Y  M  V  Y  N  F  T  D  N  S  E  E  A  A  Y  T  F  R  M  T  D  K  S  I  Y  S  R  L  I  I  S  N  D  E  Y  L  A    280
AAGTTGAGTTACATGGTGTACAACTTCACAGATAATAGTGAGGAGGCTGCTTATACATTTCGAATGACCGACAAGAGCATCTACTCGAGATTGATAATAAGTAACGATGAGTATTTGGCG   840

R  L  T  F  T  P  T  S  W  E  W  N  L  F  W  T  S  P  E  E  P  E  C̄  D  V  Y  K  T  C̄  G  S  Y  A  Y  C̄  D  V  N  T  S    320
CGACTAACGTTCACTCCGACATCATGGGAATGGAACTTGTTCTGGACTTCACCAGAGGAGCCGGAGTGCGATGTGTACAAGACTTGTGGGTCTTATGCTTACTGTGACGTGAACACATCA   960

P  V  C̄  N  C̄  I  Q  G  F  K  P  F  N  M  Q  Q  W  E  L  R  V  W  A  G  G  C̄  I  R  R  T  R  L  S  C̄  N  G  D  G  F  T    360
CCAGTGTGTAACTGTATCCAAGGTTTCAAGCCCTTCAATATGCAGCAGTGGGAACTGAGAGTCTGGGCAGGTGGGTGTATAAGGAGGACGCGGCTTAGCTGCAATGGAGATGGTTTTACC  1080

R  M  K  N  M  K  L  P  E  T  T  M  A  I  V  D  R  S  I  G  R  K  E  C̄  K  K  R  C̄  L  S  D  C̄  N  C̄  T  A  F  A  N  A    400
AGGATGAAAAATATGAAGTTGCCAGAAACTACGATGGCTATTGTCGACCGCAGTATTGGTCGGAAAGAATGTAAGAAGAGGTGCCTTAGCGATTGTAATTGTACCGCGTTTGCAAATGCG  1200

D  I  R  N  G  G  S  G  C̄  V  I  W  T  G  E  L  E  D  I  R  N  Y  F  D  D  G  Q  D  L  Y  V  R  L  A  A  A  D  L  V  K    440
GATATCCGGAATGGTGGGTCGGGTTGTGTGATTTGGACAGGAGAGCTTGAGGATATCCGGAATTACTTTGATGACGGTCAAGATCTTTATGTCAGATTGGCTGCCGCTGATCTCGTTAAA  1320

K  R  N  A  N  G  K  T  I  A  L  I  V  G  V  C  V  L  L  L  M  I  M  F  C  L  W  K  R  K  Q  K  R  A  K  T  T  A  T  S    480
AAGAGAAACGCGAATGGGAAAACCATAGCGTTGATTGTTGGAGTTTGTGTTCTGCTTCTTATGATCATGTTCTGCCTCTGGAAAAGGAAACAAAAGCGAGCAAAAACAACTGCAACATCT  1440
```

FIG. 4B

```
I  V  N  R  Q  R  N  Q  D  L  L  M  N  G  M  I  L  S  S  K  R  Q  L  P  I  E  N  K  T  E  E  L  E  L  P  L  I  E  L  E    520
ATTGTAAATCGACAGAGAAACCAAGATTTGCTAATGAACGGGATGATACTATCAAGCAAGAGACAGTTGCCTATAGAGAACAAAACTGAGGAATTGGAACTTCCATTGATAGAGTTGGAA  1560

A  V  V  K  A  T  E  N  F  S  N  C  N  K  L  G  Q  G  G  F  G  I  V  Y  K  G  R  L  L  D  G  Q  E  I  A  V  K  R  L  S    560
GCTGTTGTCAAAGCCACCGAAAATTTCTCCAATTGTAACAAACTCGGACAAGGTGGTTTCGGTATTGTTTACAAGGGTAGATTACTTGATGGGCAAGAAATTGCGGTAAAAAGGCTATCA  1680

K  T  S  V  Q  G  T  G  E  F  M  N  E  V  R  L  I  A  R  L  Q  H  I  N  L  V  R  I  L  G  C  C  I  E  A  D  E  K  M  L    600
AAAACGTCGGTTCAAGGGACTGGTGAGTTTATGAATGAGGTGAGATTGATCGCGAGGCTTCAGCATATAAACCTTGTCCGAATTCTTGGCTGTTGCATTGAGGCAGACGAGAAGATGCTG  1800

V  Y  E  Y  L  E  N  L  S  L  D  S  Y  L  F  G  N  K  R  S  S  T  L  N  W  K  D  R  F  N  I  T  N  G  V  A  R  G  L  L    640
GTATATGAGTATTTAGAAAATTTAAGCCTCGATTCTTATCTCTTCGGAAATAAACGAAGCTCTACGTTAAATTGGAAGGACAGATTCAACATTACCAATGGTGTTGCTCGAGGACTTTTA  1920

Y  L  H  Q  D  S  R  F  R  I  I  H  R  D  M  K  V  S  N  I  L  L  D  K  N  M  T  P  K  I  S  D  F  G  M  A  R  I  F  A    680
TATCTTCATCAAGACTCACGGTTTAGGATAATCCACAGAGATATGAAAGTAAGTAACATTTTGCTTGATAAAAATATGACACCAAAGATCTCGGATTTTGGGATGGCCAGAATCTTTGCA  2040

R  D  E  T  E  A  N  T  R  K  V  V  G  T  Y  G  Y  M  S  P  E  Y  A  M  D  G  V  F  S  E  K  S  D  V  F  S  F  G  V  I    720
AGGGACGAGACTGAAGCTAACACAAGGAAGGTGGTCGGAACTTACGGCTACATGTCTCCGGAGTACGCAATGGATGGGGTATTCTCGGAAAAATCAGATGTTTTCAGTTTTGGAGTCATT  2160

V  L  E  I  V  S  G  K  R  N  R  G  F  Y  N  L  N  H  E  N  N  L  L  S  Y  V  W  S  H  W  T  E  G  R  A  L  E  I  V  D    760
GTTCTTGAAATTGTTAGTGGAAAAAGGAACAGAGGATTCTACAACTTGAACCACGAAAACAATCTTCTAAGCTATGTATGGAGTCACTGGACGGAGGGAAGAGCGCTAGAAATTGTTGAT  2280

P  V  I  V  D  S  L  S  S  L  P  A  T  F  Q  P  K  E  V  L  K  C  I  Q  I  G  L  L  C  V  Q  E  R  A  E  H  R  P  T  M    800
CCAGTCATCGTAGATTCATTGTCATCATTACCAGCAACCTTTCAACCAAAAGAAGTTCTAAAATGCATACAAATTGGTCTCTTGTGTGTTCAAGAACGTGCAGAGCATAGACCAACGATG  2400

S  S  V  V  W  M  L  G  S  E  A  T  E  I  P  Q  P  T  P  P  G  Y  S  L  G  R  S  P  Y  E  N  N  P  S  S  S  R  H  C  D    840
TCGTCCGTGGTTTGGATGCTTGGAAGTGAAGCAACAGAGATTCCTCAGCCTACACCGCCAGGTTATTCCCTCGGAAGAAGTCCTTATGAAAATAATCCTTCATCAAGTAGACATTGCGAC  2520

D  D  E  S  W  T  V  N  Q  Y  T  C  S  D  I  D  A  R  *
GACGACGAATCCTGGACGGTGAACCAGTACACCTGCTCAGACATCGATGCCCGGTAGTACGAAATCCGTTGAGAAAGTTCAGATAATTAACTATTGGGGTGACCGGATATTATAAGTGAA  2640

AGAAAATAAAATTTCAATAGTTAAGTTTGTTATTTGATAACCAAATCTTGTTATTTCCTGGTGGTGTTGTCATATTCGTTTTTCTGAATGAATGTTAAAGTTATTATTC  2749
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FIG. 5

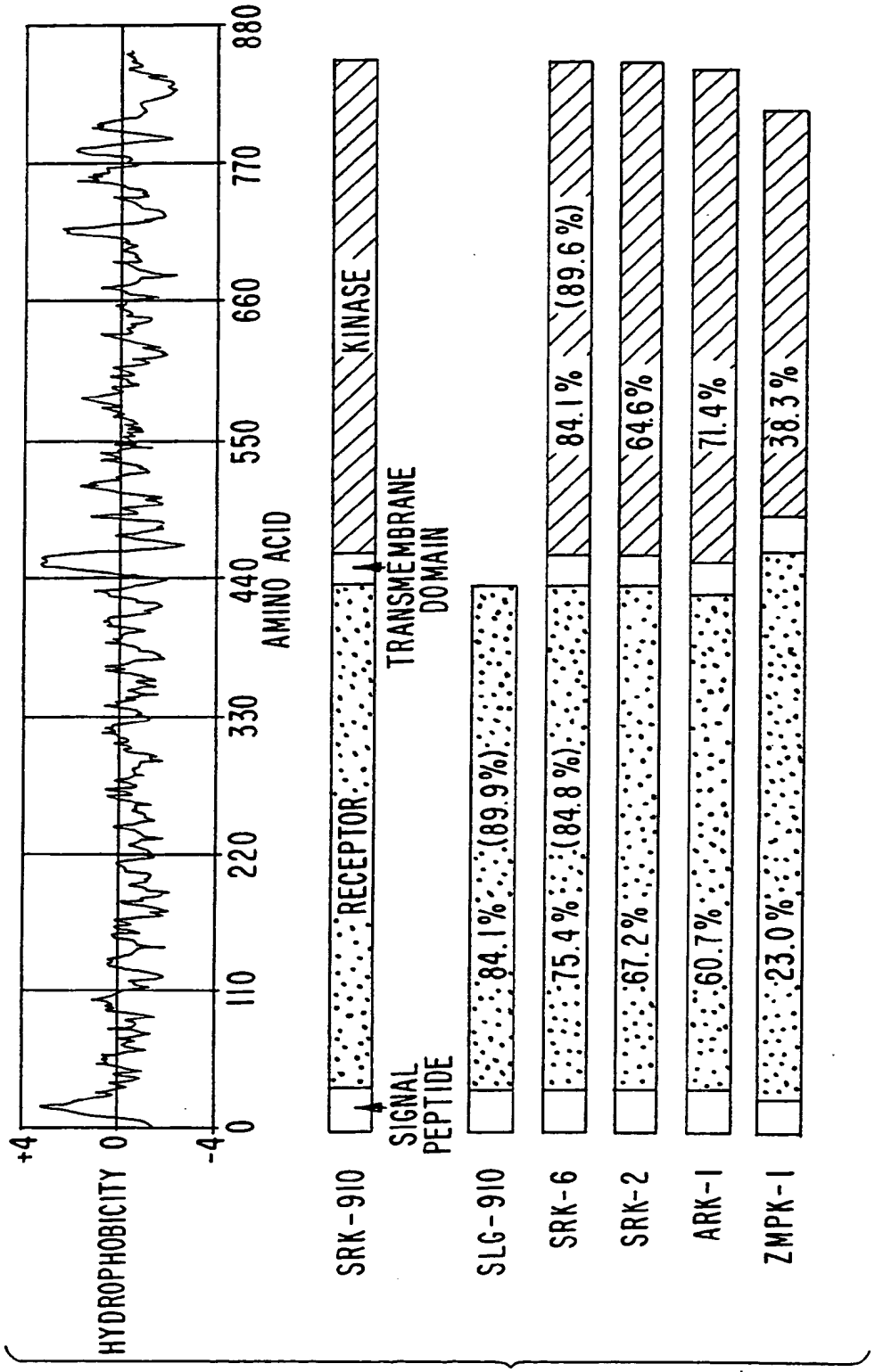


FIG. 6

```
Cons.     --------LG-G--G-V-------  ----A-K-L-----  --------E--------  ----L---------
SRK-910   ENFSNCNKLGQGGFGIVYKGRLLD  GQEIAVKRLSKTSV  QGTGEFMNEVRLIARL  QHINLVRILGCCIE 594
SRK-6     ENFSsCNKLGQGGFGIVYKGRLLD  GkEIAVKRLSKTSV  QGTdEFMNEVtLIARL  QHINLVqvLGCCIE
SRK-2     EHFSdfNKvGkGGFGvVYKGRLvD  GQEIAVKRLSemSa  QGTdEFMNEVRLmqsf  sHnNLVRlLGCCvy
ARK-1     nNFSNdNKLGQGGFGIVYKGRLLD  GkEIAVKRLSKmSs  QGTdEFMNEVRLIAkL  QHINLVRlLGCCvd
ZMPK-1    rkF  kveLGrGesGtVYKGvLeD  drhvAVKkLenvr   QGkevFqaElsvIgRi  nHmNLVRIwGfCsE
                 Domain I             Domain II        Domain III       Domain IV

Cons.     -------------------------  ------------------G--YL---------H-DLKPENI------
SRK-910   ADEKMLVYEYLENLSLDSYLFGNKR  SSTLNWKDRFNITNGVARGLLYLHQDSRFRIIHRDMKVSNILLDKNM 667
SRK-6     gDEKMLiYEYLENLSLDSYLFGktR  rSkLNWneRFdITNGVARGLLYLHQDSRFRIIHRDlKVSNILLDKNM
SRK-2     egEKiLiYEYLENLSLDShLFdetR  ScmLNWqmRFnIiNGiARGLLYLHQDSRFRIIHRDlKaSNvLLDKdM
ARK-1     kgEKMLiYEYLENLSLDShLFdqtR  SSnLNWqkRFdIiNGiARGLLYLHQDSRcRIIHRDlKaSNvLLDKNM
ZMPK-1    gshrlLVsEYvENgSLaniLFsegg  nilLdWegRFNIalGVAkGLaYLHheclewvIHcDvKpeNILLDqaf
                    Domain V                                  Domain VI

Cons.     ---I-DFG------------  ------GT--Y-APE-  ----------D-FS-GV-----------------
SRK-910   TPKISDFGMARIFARDETEA  NTRKVVGTYGYMSPEY  AMDGVFSEKSDVFSFGVIVLEIVSGKRNRGFYN 735
SRK-6     iPKISDFGMARIFeRDETEA  NTmKVVGTYGYMSPEY  AMyGiFSEKSDVFSFGVIVLEIVSGKkNRGFYN
SRK-2     TPKISDFGMARIFgRDETEA  dTRKVVGTYGYMSPEY  AMnGtFSmKSDVFSFGVllLEIiSGKRNkGlcd
ARK-1     TPKISDFGMARIFgReETEA  NTRrVVGTYGYMSPEY  AMDGiFSmKSDVFSFGVllLEIiSGKRNkGFYN
ZMPK-1    ePKItDFGlvkllnRggstq  NvshVrGTlGYiaPEw  vsslpitaKvDVySyGVvlLElltGtRvselvgg
              Domain VII           Domain VIII                  Domain IX

Cons.     ---------------------------------------------  ---------L---------R----------
SRK-910   LNHENNLL SYVW  SHWTEGRALEIVDPVIVDSLSSLPATFQPK  EVLKCIQIGLLCVQERAEHRPTMSSVVWML 807
SRK-6     LdyENdLL SYVW  SrWkEGRALEIVDPVIVDSLSSqPsiFQPq  EVLKCIQIGLLCVQElAEHRPaMSSVVWMf
SRK-2     sdsslNLL gcVW  rnWkEGqgLEIVDkVIiD  SSsP TFrPr  EiLrClQIGLLCVQERvEdRPmMSSVVlML
ARK-1     sNrdlNLL gfVW  rHWkEGneLEIVDPiniDSLSS   kFpth  EiLrCIQIGLLCVQERAEdRPvMSSVmvML
ZMPK-1    tdevhsmLrklVrmlSaklEGeeqswiDgyldskLnrpvnyvQar  tliK   lavsCleEdrskRPTMehaVqtL
                                  Domain X                        Domain XI
```

FIG. 7C

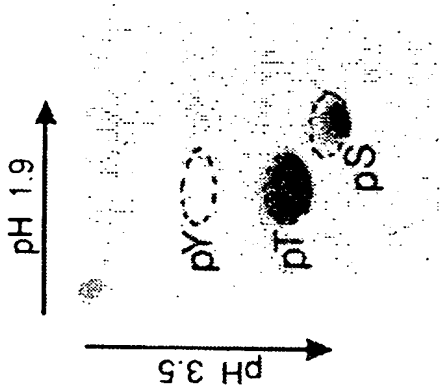


FIG. 7B

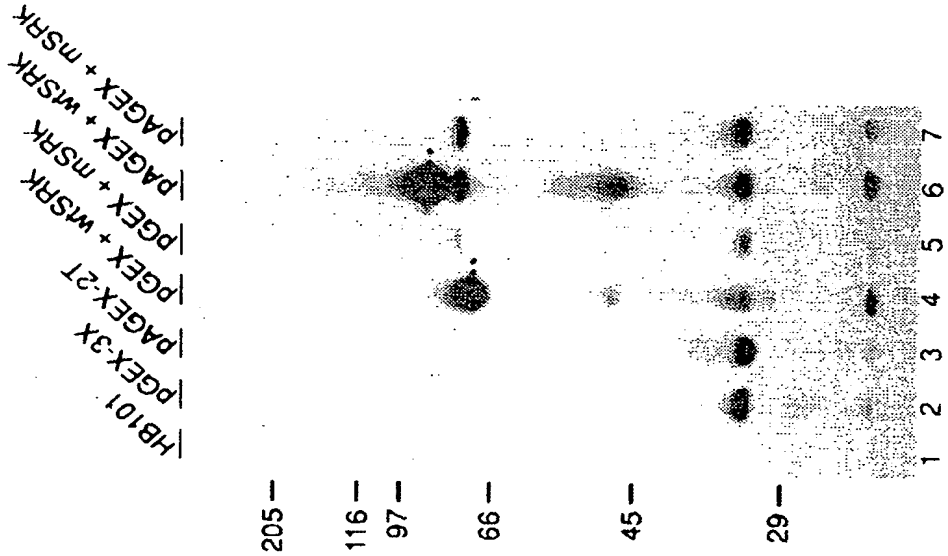


FIG. 7A

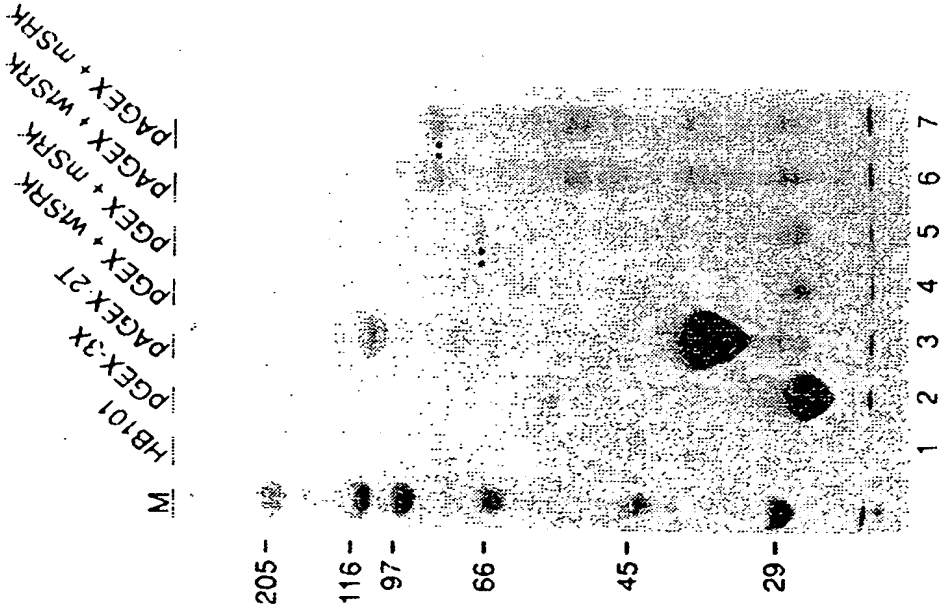


FIG. 8A

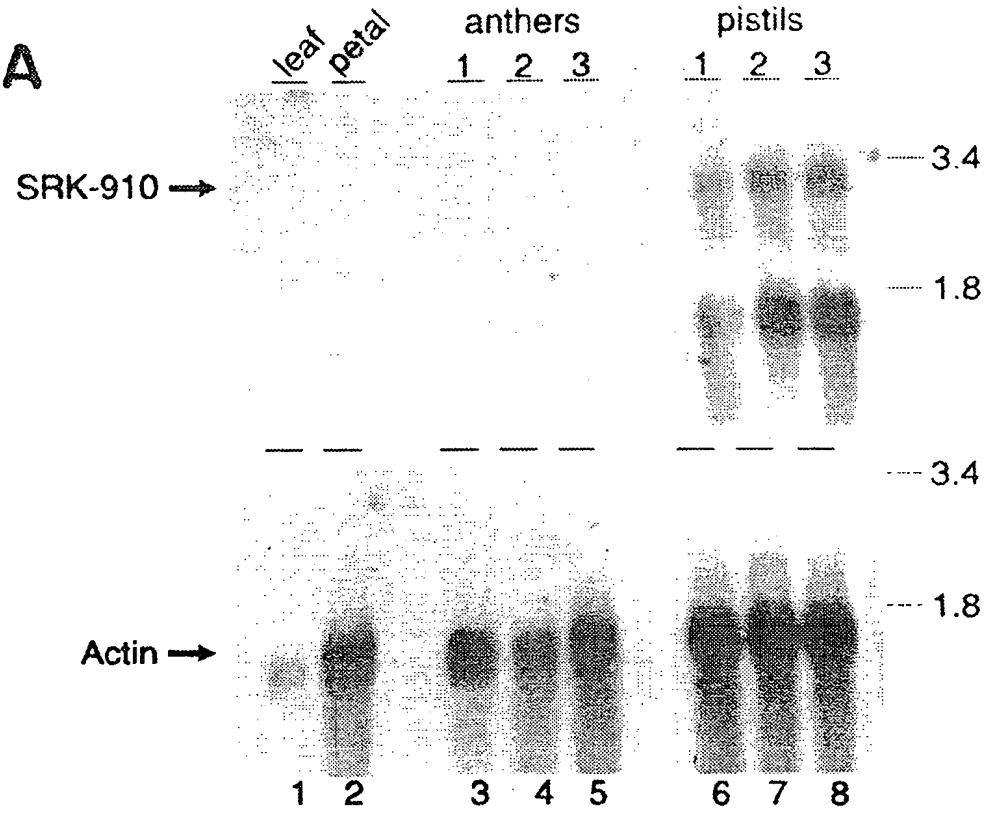


FIG. 8B

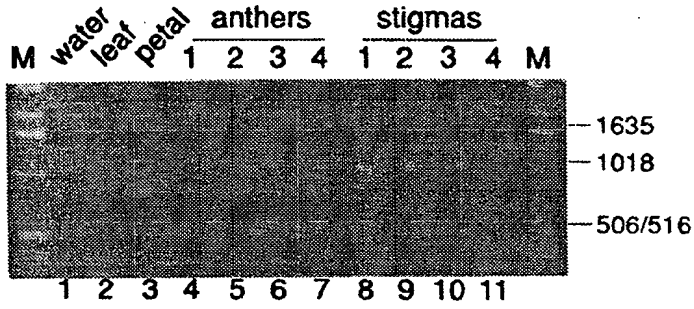


FIG. 8C

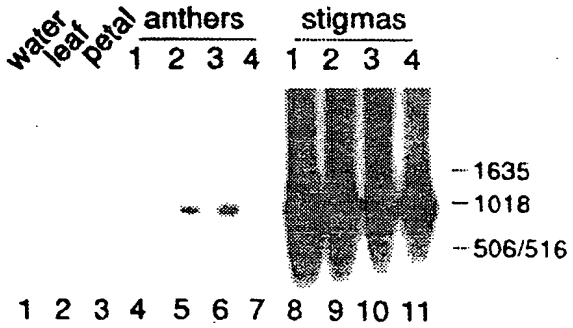


FIG. 9A

```
Met Lys Gly Val Arg Lys Thr Tyr Asp Ser Ser Tyr Thr Leu Ser Phe
 1               5                  10                  15

Leu Leu Val Phe Phe Val Met Phe Leu Phe His Pro Ala Leu Ser Ile
            20                  25                  30

His Ile Asn Thr Leu Ser Ser Thr Glu Ser Leu Thr Ile Ser Asn Asn
        35                  40                  45

Arg Thr Leu Val Ser Pro Gly Asn Val Phe Glu Leu Gly Phe Phe Arg
    50                  55                  60

Thr Thr Ser Ser Ser Arg Trp Tyr Leu Gly Ile Trp Tyr Lys Asn Leu
65                  70                  75                  80

Pro Tyr Lys Thr Tyr Val Trp Val Ala Asn Arg Asp Asn Pro Leu Ser
                85                  90                  95

Asp Ser Ile Gly Thr Leu Lys Ile Ser Asn Met Asn Leu Val Leu Leu
            100                 105                 110

Asp His Ser Asn Lys Ser Val Trp Ser Thr Asn Leu Thr Arg Gly Asn
        115                 120                 125

Glu Arg Ser Pro Val Val Ala Glu Leu Leu Glu Asn Gly Asn Phe Val
    130                 135                 140

Ile Arg Tyr Ser Asn Asn Asn Asn Ala Ser Gly Phe Leu Trp Gln Ser
145                 150                 155                 160

Phe Asp Phe Pro Thr Asp Thr Leu Leu Pro Glu Met Lys Leu Gly Tyr
                165                 170                 175

Asp Arg Lys Lys Gly Leu Asn Arg Phe Leu Thr Ala Trp Arg Asn Ser
            180                 185                 190

Asp Asp Pro Ser Ser Gly Glu Ile Ser Tyr Gln Leu Asp Thr Gln Arg
        195                 200                 205

Gly Met Pro Glu Phe Tyr Leu Leu Lys Asn Gly Val Arg Gly Tyr Arg
    210                 215                 220

Ser Gly Pro Trp Asn Gly Val Arg Phe Asn Gly Ile Pro Glu Asp Gln
225                 230                 235                 240

Lys Leu Ser Tyr Met Val Tyr Asn Phe Thr Asp Asn Ser Glu Glu Ala
                245                 250                 255
```

FIG. 9B

Ala Tyr Thr Phe Arg Met Thr Asp Lys Ser Ile Tyr Ser Arg Leu Ile
260 265 270

Ile Ser Asn Asp Glu Tyr Leu Ala Arg Leu Thr Phe Thr Pro Thr Ser
275 280 285

Trp Glu Trp Asn Leu Phe Trp Thr Ser Pro Glu Glu Pro Glu Cys Asp
290 295 300

Val Tyr Lys Thr Cys Gly Ser Tyr Ala Tyr Cys Asp Val Asn Thr Ser
305 310 315 320

Pro Val Cys Asn Cys Ile Gln Gly Phe Lys Pro Phe Asn Met Gln Gln
325 330 335

Trp Glu Leu Arg Val Trp Ala Gly Gly Cys Ile Arg Arg Thr Arg Leu
340 345 350

Ser Cys Asn Gly Asp Gly Phe Thr Arg Met Lys Asn Met Lys Leu Pro
355 360 365

Glu Thr Thr Met Ala Ile Val Asp Arg Ser Ile Gly Arg Lys Glu Cys
370 375 380

Lys Lys Arg Cys Leu Ser Asp Cys Asn Cys Thr Ala Phe Ala Asn Ala
385 390 395 400

Asp Ile Arg Asn Gly Gly Ser Gly Cys Val Ile Trp Thr Gly Glu Leu
405 410 415

Glu Asp Ile Arg Asn Tyr Phe Asp Asp Gly Gln Asp Leu Tyr Val Arg
420 425 430

Leu Ala Ala Ala Asp Leu Val Lys Lys Arg Asn Ala Asn Gly Lys Thr
435 440 445

Ile Ala Leu Ile Val Gly Val Cys Val Leu Leu Leu Met Ile Met Phe
450 455 460

Cys Leu Trp Lys Arg Lys Gln Lys Arg Ala Lys Thr Thr Ala Thr Ser
465 470 475 480

Ile Val Asn Arg Gln Arg Asn Gln Asp Leu Leu Met Asn Gly Met Ile
485 490 495

Leu Ser Ser Lys Arg Gln Leu Pro Ile Glu Asn Lys Thr Glu Glu Leu
500 505 510

Glu Leu Pro Leu Ile Glu Leu Glu Ala Val Val Lys Ala Thr Glu Asn
515 520 525

Phe Ser Asn Cys Asn Lys Leu Gly Gln Gly Gly Phe Gly Ile Val Tyr
530 535 540

Lys Gly Arg Leu Leu Asp Gly Gln Glu Ile Ala Val Lys Arg Leu Ser
545 550 555 560

FIG. 9C

```
Lys Thr Ser Val Gln Gly Thr Gly Glu Phe Met Asn Glu Val Arg Leu
                565                 570                 575

Ile Ala Arg Leu Gln His Ile Asn Leu Val Arg Ile Leu Gly Cys Cys
            580                 585                 590

Ile Glu Ala Asp Glu Lys Met Leu Val Tyr Glu Tyr Leu Glu Asn Leu
        595                 600                 605

Ser Leu Asp Ser Tyr Leu Phe Gly Asn Lys Arg Ser Ser Thr Leu Asn
    610                 615                 620

Trp Lys Asp Arg Phe Asn Ile Thr Asn Gly Val Ala Arg Gly Leu Leu
625                 630                 635                 640

Tyr Leu His Gln Asp Ser Arg Phe Arg Ile Ile His Arg Asp Met Lys
                645                 650                 655

Val Ser Asn Ile Leu Leu Asp Lys Asn Met Thr Pro Lys Ile Ser Asp
            660                 665                 670

Phe Gly Met Ala Arg Ile Phe Ala Arg Asp Glu Thr Glu Ala Asn Thr
        675                 680                 685

Arg Lys Val Val Gly Thr Tyr Gly Tyr Met Ser Pro Glu Tyr Ala Met
    690                 695                 700

Asp Gly Val Phe Ser Glu Lys Ser Asp Val Phe Ser Phe Gly Val Ile
705                 710                 715                 720

Val Leu Glu Ile Val Ser Gly Lys Arg Asn Arg Gly Phe Tyr Asn Leu
                725                 730                 735

Asn His Glu Asn Asn Leu Leu Ser Tyr Val Trp Ser His Trp Thr Glu
            740                 745                 750

Gly Arg Ala Leu Glu Ile Val Asp Pro Val Ile Val Asp Ser Leu Ser
        755                 760                 765

Ser Leu Pro Ala Thr Phe Gln Pro Lys Glu Val Leu Lys Cys Ile Gln
    770                 775                 780

Ile Gly Leu Leu Cys Val Gln Glu Arg Ala Glu His Arg Pro Thr Met
785                 790                 795                 800

Ser Ser Val Val Trp Met Leu Gly Ser Glu Ala Thr Glu Ile Pro Glu
                805                 810                 815

Pro Thr Pro Pro Gly Tyr Ser Leu Gly Arg Ser Pro Tyr Glu Asn Asn
            820                 825                 830

Pro Ser Ser Ser Arg His Cys Asp Asp Asp Glu Ser Trp Thr Val Asn
        835                 840                 845

Gln Tyr Thr Cys Ser Asp Ile Asp Ala Arg
    850                 855
```

FIG. 10

FIG. II

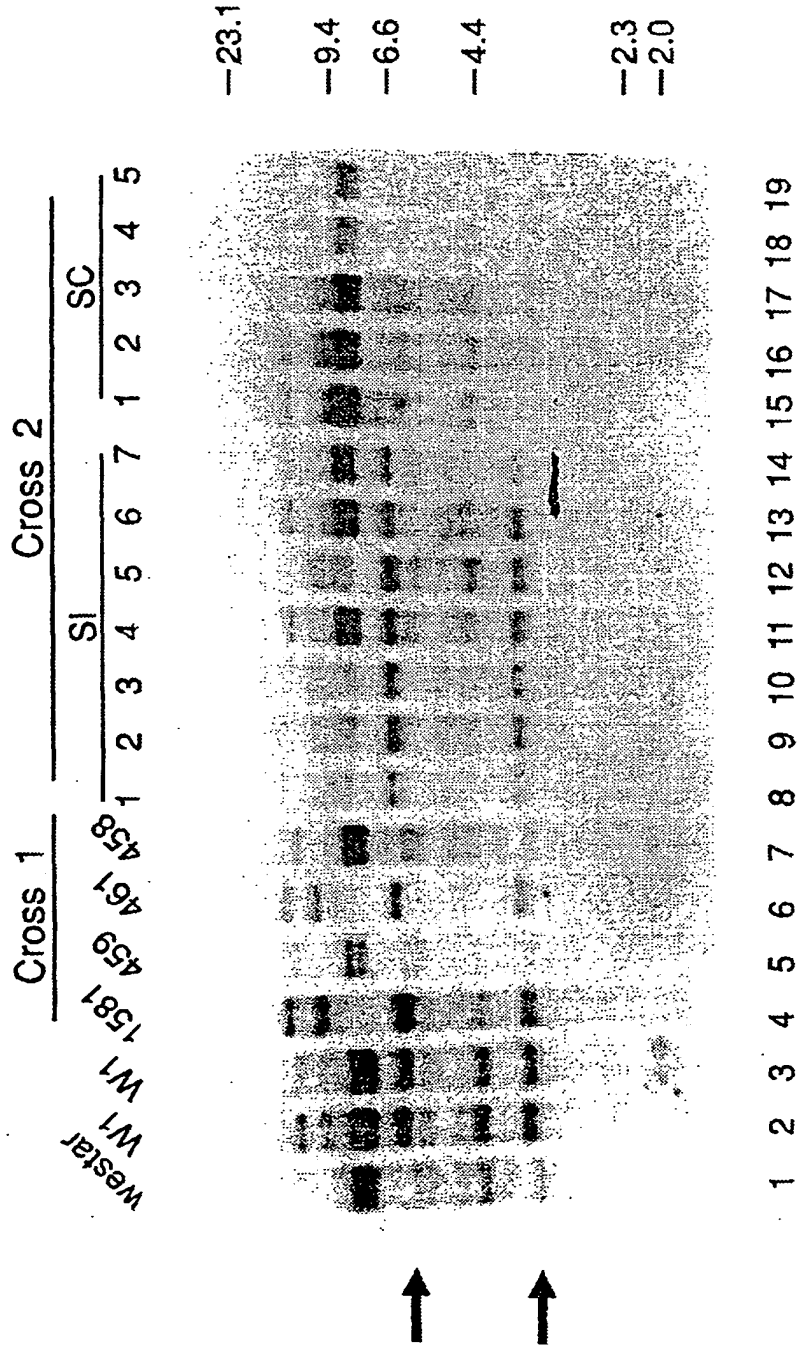
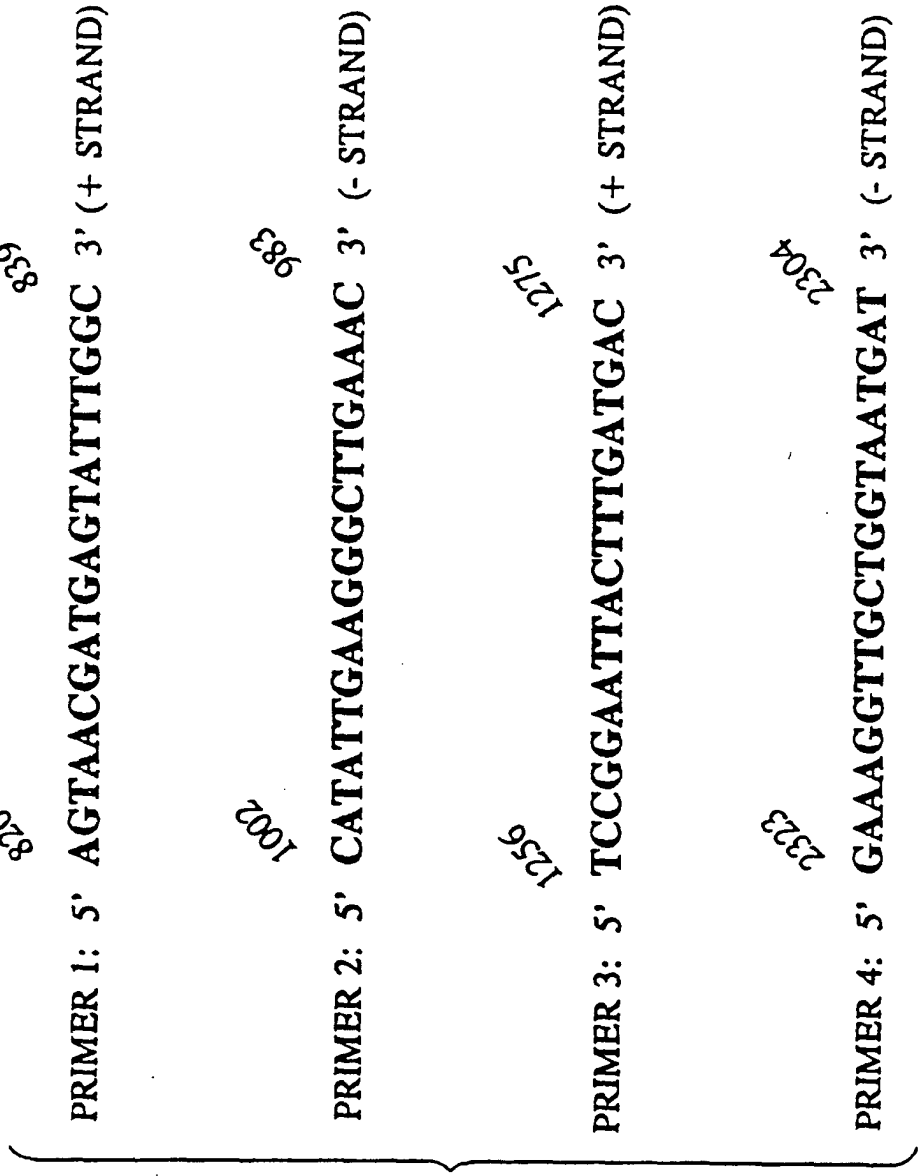


FIG. 12



INTERNATIONAL SEARCH REPORT

International Application No
US 93/09448

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/29 C12N15/82 C07K13/00 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12N C07K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EMBL SEQUENCE DATABASE. 5 October 1992 , RELEASE 33. AC M97667 GORING, D.R., ET AL. 'Brassica napus ssp. oleifera serine/threonine kinase receptor mRNA, complete cds.'	1-6
Y	see the whole document	9,14

X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA vol. 88 , October 1991 , WASHINGTON US pages 8816 - 8820 STEIN, J.C., ET AL. 'Molecular cloning of a putative receptor protein kinase gene encoded at the self-incompatibility locus of Brassica oleracea'	7
Y	see the whole document	14

	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
23 February 1994	04 -03- 1994

Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 436 467 (CIBA-GEIGY) 10 July 1991 see page 15 - page 21; claims 32,33	9,14
P,X	EP,A,0 519 869 (CIBA-GEIGY) 23 December 1992 see page 33, line 35 - line 58; claims 15,18	7,14
P,X	THE PLANT CELL. vol. 4, no. 10 , October 1992 , ROCKVILLE, MD, USA. pages 1273 - 1281 GORING, D.R., ET AL. 'The S-locus receptor kinase gene in a self-incompatible Brassica napus line encodes a functional serine/threonine kinase' see the whole document	7,9
A	MOLECULAR AND GENERAL GENETICS vol. 234, no. 2 , August 1992 , BERLIN DE pages 185 - 192 GORING, D.R., ET AL. 'Use of the polymerase chain reaction to isolate an S-locus glycoprotein cDNA introgressed from Brassica campestris into B. napus ssp. oleifera' see the whole document	10-13
A	THEOR. APPL. GENET. vol. 82 , 1991 pages 466 - 472 GUILLUY, C.-M., ET AL. 'PCR detection of transcripts homologous to the self-incompatibility gene in anthers of Brassica' see the whole document	10-13
A	WO,A,90 12097 (GENELABS) 18 October 1990 see page 30 - page 34; claim 12	10-13
A	NATURE vol. 345 , 21 June 1990 , LONDON GB pages 743 - 746 WALKER, J., ET AL. 'Relationship of a putative receptor protein kinase from maize to the S-locus glycoproteins of Brassica' see figure 1	8

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
US 93/09448

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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		AU-A-	6855490	11-07-91
EP-A-0519869	23-12-92	CA-A-	2071473	20-12-92
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		CA-A-	2050593	05-10-90
		EP-A-	0466817	22-01-92
		JP-T-	4504208	30-07-92
		US-A-	5101025	31-03-92
		US-A-	5128460	07-07-92
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